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THE JOURNAL OF HYGIENE

EDITED BY

GEORGE H. F. NUTTALL, M.D., Ph.D., Sc.D., F.R.S.
QUICK PROFESSOR OF BIOLOGY IN THE UNIVERSITY OF CAMBRIDGE

IN CONJUNCTION WITH

JOHN S. HALDANE, M.D., LL.D., F.R.S.
LATE READER IN PHYSIOLOGY IN THE UNIVERSITY OF OXFORD

CHARLES J. MARTIN, M.B., D.Sc., F.R.S.
DIRECTOR OF THE LISTER INSTITUTE, LONDON

J. C. G. LEDINGHAM, M.B., D.Sc.
BACTERIOLOGIST-IN-CHIEF, LISTER INSTITUTE, LONDON

G. S. GRAHAM-SMITH, M.D.
UNIVERSITY LECTURER IN HYGIENE, CAMBRIDGE

M. GREENWOOD, Junr., M.R.C.S.
STATISTICIAN, LISTER INSTITUTE, LONDON

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TRANSMISSION OF PULMONARY AND SEPTICAEMIC PLAGUE AMONG MARMOTS[1].

By WU LIEN-TEH, M.A., M.D. (Cantab.),
Director and Chief Medical Officer,

and FREDERICK EBERSON, M.A. (Columbia), M.S. (Iowa),
Bacteriologist, North Manchurian Plague Prevention Service.

The subject of plague transmission among marmots was brought up at the time of the historic epidemic of pneumonic plague which raged in Manchuria and Northern China during the winter of 1910–1911. The tarbagan (*Arctomys bobac*) was suspected of having played a part in the transmission of the disease, yet no experimental evidence was brought forth to show that this animal was in any way associated with plague. One of the present writers undertook an expedition to the tarbagan regions in Mongolia in 1911, and in a paper published in this *Journal*[2] produced ample evidence to show that under normal conditions plague did not exist among the marmots. It was Strong[3], working in Mukden, who first performed experiments of plague inhalation upon tarbagans, and published data concerning the possible importance of these animals in the epidemiology of this dread disease. He demonstrated, in a general way, that they could take pneumonic plague if exposed to the organisms sprayed in droplet form.

The present enquiry has been undertaken to elucidate this point clearly and to determine the part played by the marmot in the spread of infection through contact and feeding on plague corpses. The animals used were a small species related to the tarbagan and known as *Spermophilus citellus*. These ferret-like creatures are very numerous in the Mukden district and can be found in larger numbers in the summer

[1] The experiments here summarised were conducted at the Mukden Medical College. To Dr Dugald Christie, C.M.G., the Principal, and other members of the staff we are greatly indebted for their kindness and courtesy in providing us with facilities for our work.

[2] Wu Lien-Teh (1913) *Journ. of Hygiene*, XIII. 237.

[3] Strong (1912) *Philippine Journ. Sci.* VII.

season. They frequent the graveyards and burrow underground, not unlike the ground squirrel or American gopher. In size, they approximate to the rat, are very vicious and make good use of their long and exceedingly sharp teeth and claws. They can be readily trapped by pouring water into their burrows, when they rush out to escape suffocation. With care in handling, especially while trapping, they will live in captivity for a long time.

Our present work on Marmots may be conveniently grouped under experiments relating to: (A) Infection by Inhalation; (B) Susceptibility to Infection by Subcutaneous Inoculation; (C) Infection by Feeding; and (D) Histological Examination.

A. Inhalation Experiments.

The method used in these experiments conformed as far as possible to the natural conditions for infection with pulmonary plague. A 24-hour old agar slant of a moderately virulent strain of *B. pestis* was suspended in 10 c.c. of salt solution and sprayed from a graduated cylinder fitted with a very fine nozzle. Great care was taken to direct the spray in a fine cloud towards but not into the nasal passage. Although a definite amount of culture was used in the two different series of experiments, it was not possible to determine more than roughly, if at all, how much of the culture found its way into the respiratory tract. In the first series, five minims were sprayed, and in the second, ten minims. A great portion of the spray obviously failed to inoculate, because of the mechanical difficulties entailed in the spraying technique and of the position of the animals on the stage. It was necessary to clamp the neck in order to hold the animals down properly, and in many instances their normal breathing was impeded. The errors due to the loss of most of the culture spray tend to make the results obtained more striking. Before spraying, the animals were covered with a piece of absorbent cotton soaked in cresol lotion, so as to prevent droplets lodging on the fur, and after exposure to the spray the head of each marmot was carefully wiped to remove extraneous organisms. In order to minimise the danger from droplet infection, a specially constructed wooden box was used while the animal was being sprayed. This box enabled the operator to place the animal and stage in a compartment with a glass top, and permitted the spray to be introduced through an opening at the front of the box. This aperture was on a level with the animal's head as it lay fastened on the stage, and the spray, directed through the opening reached the nose quite readily.

Series I.

In this series the animals were placed in flea-proof cages, 20 ins. long by 15 ins. wide by 9 ins. high, separated from the normal animals in some instances by a single partition and in others by a complete four-walled compartment which stood in the centre of the cage. This latter type of cage was used in order to enable the sprayed animals to run about the central compartment, thus permitting them to come in freer intercourse with others outside, herein called contacts. Five minims of the culture in salt solution were sprayed in each case.

Exp. No.	Contact animal's No.	When placed in contact	Result	Sprayed animal's No.	Result
1	1	Same day	12 days. No plague	1	Killed, 20th day. No plague
	2	,,	Killed, 21st day. No plague	2	Killed, 20th day. No plague
2	1	,,	7 days. Typical plague pneumonia	1	12 days. Typical plague pneumonia
	2	,,	7 days. Typical plague pneumonia	2	Killed, 18th day. No plague
3	1	one day after	Alive, well, 22nd day	1	10 days. Typical plague pneumonia
	2	,,	Alive, well, 22nd day	2	Alive, well, on 22nd day
4	1	2 days after	15 days. No plague	1	5 days. Typical plague pneumonia
	2	,,	Alive, well, 22nd day	2	7 days. Typical plague pneumonia
				3	7 days. Typical plague pneumonia
5	1	3 days after	3 days. No plague	1	5 days. Typical plague pneumonia
	2	,,	7 days. Typical plague pneumonia	2	7 days. Typical plague pneumonia
				3	7 days. Typical plague pneumonia
Total	10		3	12	8

In Exp. 1 no changes were noted in the organs of the animals which died or which were killed. Smears and cultures from the organs and blood were uniformly negative. In Exp. 2 it is interesting to note that a "contact" succumbed five days before any of the sprayed animals died, and that out of the latter two one lived for 12 days, while a mate, confined in the same cage, failed to take plague. It is very evident that here, as with man, a difference in resistance prevails. Apart from this we must take into account the different factors which tend to modify the chances for infection even by close contact. Exp. 3 offers an example

of plague in a chronic form. This first series of experiments indicates that pneumonic plague can be readily transmitted to the marmot, and that animals suffering from plague pneumonia in turn are capable of transmitting the disease to others. In Exp. 5 it should be noted that one animal proved infective to a normal contact as early as two days after inhaling plague bacilli.

Summarising the results obtained, we may say that the chances for infection by contact were undoubtedly minimised by our method of housing the animals, therefore the few positive cases which occurred tend to render the results all the more surprising. Out of 12 animals exposed to infection by inhalation, eight (66·6 %) died of typical plague pneumonia in from three to seven days. Of the contacts, three (30 %) died and seven survived. Fifty per cent. of the animals which were exposed directly or indirectly to the disease died.

In order to ensure more natural living conditions, such as normally prevail among these animals, a second series of experiments was conducted with the contacts placed in unscreened cages. Careful examination had already revealed that fleas were very scarce on the marmots at the time of our visit (July-August) and whatever insect transmission might occur would be easily recognised from the resultant type of plague. This phase of the problem is now being studied and will form the subject of a separate report.

Series II.

Exp. No.	Contact animal's No.	When placed in contact	Died	Sprayed animal's No.	Died
1	1	1 day after	5 days. Typical plague pneumonia	1	3 days. Typical plague pneumonia
	2	..	12 days. Typical plague pneumonia	2	9 days. Typical plague pneumonia
	3	..	12 days. Typical plague pneumonia		
2	1	2 days after	4 days. Typical plague pneumonia	1	5 days. Typical plague pneumonia
	2	..	6 days. Typical plague pneumonia	2	Killed, 17th day. No plague
3	1	3 days after	5 days. Typical plague pneumonia	1	5 days. Typical plague pneumonia
	2	..	6 days. Typical plague pneumonia	2	6 days. Typical plague pneumonia
4	1	4 days after	Alive, well, 16th day	1	15 days. No plague
	2	..	Alive, well, 16th day		
Total	9		7	7	5

A summary of the results obtained in this series of experiments shows in a conclusive manner that pneumonic-plague-infected marmots can readily transmit the infection through the respiratory passages as in the case of man. Conditions which favour the propagation of the disease among the latter are in no way different for these animals. Close contact and moist surroundings seem to favour the rapid spread of infection from animal to animal. Out of seven marmots inoculated by inhalation, five (71 %) died after 4–6 days with acute pneumonic plague and septicaemia. Nine contacts placed with infected animals after periods varying from 1–4 days showed a mortality of 77 %. These seven marmots died after 4–6 days' contact. Here also, as in the preceding series, was noted a remarkably short incubation period, with marked infectivity on the part of the sprayed animals.

Summary.

First Series.				Second Series.			
Animals inoculated	12	Deaths	8	Animals inoculated	7	Deaths	5
Contacts ...	10	,,	3	Contacts	9	,,	7
Total	22	,,	11	Total	16	,,	12

Animals exposed to plague	38
Deaths	23

The autopsy findings indicate clearly that pneumonic plague among marmots is not unlike that in man. The bacilli, entering the respiratory tract, lodge in the lungs and from this primary focus enter the circulation and cause a general septicaemia. The pathological changes in the organs are most striking, they chiefly affect the lungs. The latter show extreme congestion and inflammation with fibrinous exudation, and, associated therewith, pronounced pleuritis. Enlargement of the spleen and liver is frequent, though not constant, and visceral congestion is prominent. No instance of axillary or inguinal gland involvement were observed, although in a few cases the cervical glands were enlarged, and, upon microscopical examination of smears, showed plague bacilli in good numbers. Inflammation of the trachea and bronchi occurs with marked regularity.

Bacteriological examinations demonstrated that although the lungs may contain enormous numbers of plague bacilli, yet this organ is not exclusively selective. In a fair proportion of the cases where the lung showed few organisms the spleen invariably teemed with them. This was also noted when blood smears were not particularly full of *B. pestis*. The number of organisms present in any one organ at a given time seems

to depend upon a variety of circumstances, not the least of which appears to be the individual resistance of the animal. Some animals, it was noted, may show a distinct toxaemía without marked bacteraemia. In analogous fashion, a few of the marmots may offer such low resistance to the disease that they succumb before any very marked changes appear in the organs.

Of great interest is the observation that plague may exist in a chronic form among marmots. That they can live for nine or ten days with pronounced plague and be capable of conveying infection to other animals, is of the utmost importance from an epidemiological standpoint.

B. Susceptibility to Infection.

Plague septicaemia, as seen above, results readily from plague pneumonia. In order to study the susceptibility of marmots to this type of plague, a number of animals were inoculated subcutaneously with varying doses of bacilli. All the animals died of acute septicaemic plague with slight, if any, signs of bubonic affection. This experiment shows that marmots are very susceptible to plague septicaemia. The culture used was only moderately virulent, and had been growing on agar for several generations. A 72-hour growth on small agar slants was used for inoculation.

Marmot No.	Dose (slant)	Result	Postmortem findings
1	1/20	Death, 2 days	Intense congestion at site of inoculation. Spleen and liver enlarged and congested. Smears from blood and organs showed enormous numbers of plague bacilli.
2	1/40	„ 2 days	Ditto
3	1/80	„ 6 days	„
4	1/40	„ 4 days	„
5	1/80	„ 5 days	„
6	1/80	„ 36 hours	„
7	1/80	„ 36 hours	„
8	1/80	„ 48 hours	„
9	1/80	„ 48 hours	„
10	1/80	„ 48 hours	„

Animals No. 6 to No. 8 inclusive were inoculated with the same strain after a single passage through a marmot.

C. Plague Transmission by Feeding.

Group I. Three marmots were fed with liver and spleen taken from a guinea-pig which had died of plague after 56 hours.

Marmot 1. Died after 3 days. Liver and spleen congested. Stomach inflamed. Smears from lungs, liver, spleen and blood showed *B. pestis*. A blood culture injected into a normal marmot killed the animal within 36 hours. Acute plague with *B. pestis* in the blood and all organs.

Marmot 2. Died after 3 days. This animal was eaten by the remaining marmot before an autopsy could be made.

Marmot 3. Died after 4 days. Slight visceral congestion. Inflammation of the gastric mucosa. Smears from blood and organs showed *B. pestis*. The stomach scrapings were full of plague bacilli.

Group II. Three marmots were fed with lung, liver and spleen taken from a marmot which had died of acute plague.

Marmot 4. Died after 2 days. Congestion of liver and spleen. Marked visceral congestion and intense inflammation and congestion of gastric mucosa. Smears from organs and stomach lining showed great numbers of *B. pestis*.

Marmot 5. Died after 4 days. Liver and spleen congested and enlarged. Visceral congestion marked. Gastric mucosa greatly inflamed. Smears from blood and organs gave enormous numbers of *B. pestis*.

Marmot 6. Killed after 14 days. Autopsy: no changes in any of the organs or glands; a very slight area of old inflammation noticed in the gastric mucosa, but no plague bacilli seen in scrapings; smears from blood and organs negative. This animal had been fed twice with plague material with an interval of one week elapsing between the feedings.

This series of experiments, though small, demonstrates that marmots may transmit plague by feeding on plague carcases. The animals are carnivorous by nature, and promptly eat their dead mates. Death after infection by feeding takes place within four days and is apparently not hastened by greater amounts of plague-infected material. The most striking change in the marmot consists in the intense inflammation of the gastric mucosa. The spleen and liver show the usual changes attendant upon plague. That individual differences in susceptibility may exist is well exemplified in the case of marmot 6, which failed to take plague although it had been given a large amount of highly infective material.

The experiments upon *Spermophilus citellus* mentioned above, particularly with regard to pulmonary plague infection by contact, are perhaps the first that have been recorded. The feeding experiments are especially interesting because many workers, including Strong, have denied the possibility of plague transmission by this means.

D. Histological Examination.

The histological changes observed in the lesions of human pulmonary plague have been fully described by various writers, including Albrecht and Gohn[1], Childe[2], Strong[3], Fujinami[4], and Wu Lien-Teh and Woodhead[5]. We preserved and examined a considerable number of specimens obtained from the animals experimented upon, but as the microscopical changes in the inhalation experiments differ in no material way from those already described in the case of human pulmonary plague, we shall only refer to them briefly.

Lung. In acute pulmonary plague (*i.e.* where the animals died in 2–5 days after infection), sections of the lung showed intense congestion of the blood vessels. The part of the lung tissue adjacent to the pleura was marked by much leucocytosis and even haemorrhage, and areas of collapse could be seen. The small bronchi were filled with mucoid substance, and some were practically choked with pure cultures of plague bacilli. Around the inflamed bronchi and bronchioles were patches of pneumonia, harbouring numbers of bacilli in the capillaries and alveoli. No fibrinous lymph coagulum was noted.

In specimens obtained from two cases which died 12 days after contact with infected animals, the lung tissue showed somewhat different changes. Here haemorrhage and congestion were not so marked, and broncho-pneumonic patches were scanty. The alveoli displayed extensive signs of collapse, and around the bronchi considerable signs of inflammation and thickening were noted. Plague bacilli were not nearly so numerous as in acute plague.

Liver. In the acute form, sections of the liver showed a picture of acute red atrophy, the central lobular vein being much distended, and the portal capillaries swollen. The hepatic cells were markedly 'cloudy' and granular, but vacuolation, except in a few areas, had not set in earnestly. Haemorrhage were noted everywhere.

In specimens obtained from the chronic cases, the liver substance showed very characteristic signs of degeneration. The central lobular

[1] Albrecht and Gohn, *Centralbl. f. Bakteriol.* 1899, xxvi. 362.

[2] Childe, *Brit. Med. Journ.* 1897, i. 1215; 1898, ii. 858, and *Report of Indian Plague Comm.* London, 1900.

[3] Strong, *Rep. Intern. Plague Conference*, Mukden, 1912.

[4] Fujinami, *Ibid.*

[5] Wu Lien-Teh and Woodhead (1913), *Journ. of Pathology and Bacteriol.* 1913.

vein was not so distended, and haemorrhage was not so marked. A large portion of the hepatic cells appeared to have lost their contents, so advanced was the vacuolation and loss of nuclear substance. In fact the whole section stained badly with haematin. Plague bacilli were seen with difficulty.

Spleen. Here also the changes observed in the acute and chronic disease were characterised by much more congestion in the former than in the latter. The number of bacilli encountered was also greater in the acute form, and the Malpighian bodies were larger and stood out more distinctly.

Kidney. As in the case of the liver, the kidney showed far more extensive signs of degeneration in the chronic than in the acute cases. There was very little thickening of the capsule in either case, but the glomeruli were swollen considerably. In the chronic cases the cells of the tubules had lost the greater part of their substance, and in several places only the basement membrane was seen, so great had been the disintegration. More haemorrhage was noted in the kidney than in other organs in the chronic cases.

Heart. The muscular tissue showed oedematous changes, the striations being more indistinct than usual, and the muscle fibres broken in places.

Lymphatic Gland. Both cervical and inguinal glands were examined, but showed no changes other than those hitherto described in ordinary plague. Plague bacilli were present in lesser numbers than in bubonic plague, and in the chronic form were sometimes not seen at all.

Stomach. So many observers have denied the existence of infection by the alimentary canal that a little more attention may be devoted to the changes observed in this organ. As stated in the preceding article, out of six marmots fed upon plague-infected viscera five died – four definitely of plague, whilst the fifth one was eaten by its fellows before an examination could be made. Only one animal survived, and after 14 days was killed, but it showed no signs of infection. Autopsies in all cases were made within a few hours after death, and the stomach of all the infected animals showed definite signs of acute inflammation, which was most marked at the pyloric end and commencement of the duodenum. Red patches denoting haemorrhage and small areas of disintegration were clearly seen. Pieces of the stomach at the pyloric end were removed from cases 4 and 5, and prepared for microscopical examination. Formalin was used as fixing agent, and the

paraffin sections were stained both with alum haematin plus eosin, and also with dilute Giemsa, as follows:

1. Stain in dilute Giemsa (1 part Giemsa solution (Grübler) in 10 parts distilled water) for 6 hours.
2. Decolorise in weak acetic acid (5 drops in 100 c.c. distilled water).
3. Wash in distilled water.
4. Blot and clear in xylol.

Plague bacilli, when present, are stained blue in the tissues by this method.

The gastric mucosa shows marked changes under the microscope. The mucous glands are intensely inflamed, and haemorrhages can be seen both inside and around them. Clots with fibrin are also encountered, sometimes firmly adherent to the underlying glands. In places large areas of glandular tissue have given way, revealing open ulcers with much leucocyte infiltration and ruptured blood vessels around the edges. Apparently the large oxyntic cells are first cast out, for here and there numbers of them are found on the surface intermixed with leucocytes. In other parts, where disintegration had been extensive, only granular débris is left. The cells of the glands are swollen and granular, and where inflammatory changes are most marked they appear broken up. Plague bacilli are met with in varying numbers amidst the glands, and are most evident on the surface of the necrotic areas. The submucous coat is thickened, the blood vessels supplying the glands being much distended and filled with corpuscles. The inner circular muscular coat is also congested, and large clumps of plague bacilli are seen distributed among the fibres, especially in the neighbourhood of blood vessels. The fibres themselves appear swollen, but no signs of disintegration can be made out. The outer longitudinal muscular coat seems also swollen, but very few bacilli are met with in this region. The peritoneal coat is slightly infiltrated in certain parts.

In the sections obtained from Marmot 5, the surface of the mucous coat seems to be largely covered with an organised coagulum of mucoid tissue of varying thickness. Where the clot had broken off, the mucous glands show necrotic changes similar to those described above, and the surrounding blood vessels are largely distended. Plague bacilli are present in large numbers both inside and outside the clot, and in the granular débris of the mucous glands.

SUMMARY AND CONCLUSIONS.

1. 52·6 % of marmots placed in contact with marmots infected with plague by inhalation developed pulmonary plague and died within 4–6 days.

2. Marmots suffering from pneumonic plague are infective at an early stage of the disease and the animals which such marmots infect acquire plague after a short incubative period.

3. Pulmonary plague can be readily transmitted to the small marmot (*Spermophilus citellus* Linn.), and these animals, when suffering from pulmonary plague, are in turn capable of transmitting the same type of plague through the respiratory passages.

4. Septicaemic plague can be developed in marmots very easily as a result of respiratory infection and also by direct subcutaneous inoculation with small amounts of culture.

5. The marmot can acquire plague by way of the alimentary tract and spread the disease by feeding on plague-infected carcases. The histological appearances observed in the lesions of these cases are characteristic.

NOTE ON ECTOPARASITES FROM MARMOTS.

Accompanying a letter dated 16th August 1916, Dr Wu Lien-Teh forwarded some specimens of ectoparasites collected by him from marmots.

1. A single flea: The specimen was determined by Hon. N. C. Rothschild as a slightly aberrant example of *Ceratophyllus famulus* Jordan and Rothschild, 1911 (*Proc. Zool. Soc. London,* 1911, p. 380, No. 7, text-fig. 115).

2. A number of ticks: The specimens consisted of but a single female accompanied by a number of nymphs, some mounted in balsam, others not. Mr C. Warburton examined the specimens and determined them as *Haemaphysalis,* one of the variants of *H. leachi,* closely resembling the form described as *H. koningsbergeri* Warburton and Nuttall, 1909 (see *Ticks, A Monograph of the Ixodoidea,* 1915, Part III, p. 468, figs. 408–410). It is desirable that more adults should be secured, including both sexes, to enable a precise determination to be made. Specimens should be preserved in 70 % spirit.

G. H. F. NUTTALL.

THE INFLUENCE OF THE AGE OF THE PARENT AT BIRTH OF CHILD ON EYE-COLOUR, STATURE AND INTELLIGENCE.

By R. J. EWART, M.D., M.Sc., F.R.C.S., D.P.H.,
Medical Officer of Health, Barking Town, E.

WE have already considered, in previous papers, the influence of parental age, on birth, marriage, sex, susceptibility, and reaction to bacterial invasion, evidence having been produced which lends support to the belief that this factor does modify the organism in its behaviour with respect to the attributes considered. It is therefore reasonable to extend the enquiry, and examine whether these variations are accompanied by modifications in structural attributes. In the present paper the characters studied are, eye-colour, stature and intelligence.

Eye-colour was selected on account of its interest and comparative ease of observation; stature as a measure of growth; and intelligence, judged by scholastic standard, as a prime factor in the success of man in the eternal conflict between man and his animate or inanimate competitors.

Eye-colour.

Any general consideration of the enormous literature treating this subject is unnecessary; the work has already been done in great detail by Pearson, Nettleship and Usher, in their monograph on Albinism. All that is needful in the present instance is a reference to such works as have a direct bearing on the points discussed.

Before considering the actual data, some comment must be made on the tacit assumption that eye colour is a fixed character, apparently not modified during the life of the individual. It should be remembered that all are born with blue eyes of varying shades, and that a proportion change to brown, grey, etc. The method by means of which this change is achieved is somewhat doubtful, and may be explained in two ways:

(*a*) by the deposit of a new layer of pigment in front of the choroidal layer, or (*b*) by the pigment already present undergoing a physical change, that is, the intracellular granules change from a molecular to a granular state. There is no histological evidence in favour of the former supposition, nor does direct observation of the iris reveal appearances which would lend credence to the belief that an anterior layer of pigment was being deposited.

The second hypothesis is in accord with the physical appearance of many substances when in different states. It is easily conceived that with such a pigment, placed in a semitranslucent or whitish stroma, with a slight tinge of red dependent on the capillary circulation, any known eye-colour could be obtained.

The rate at which this change takes place in childhood varies, and there is some evidence that even in adults the same tendency is observed.

The cause is doubtful, though the fact that the central areas of the iris are darker than the peripheral portions, does suggest that intensity of illumination is a factor in its production.

Pearson has already shown that even between the 5th and 20th years, there is a tendency for the eyes to darken in colour.

He gives the following values:

Virchow's data.	Age and	Eye-colour		$C_r = \cdot 027$
Pletgour's	,,	,,	,,	$C_r = \cdot 451$
Urchida	,,	,,	,,	$C_r = \cdot 096$

The values seem to vary according to the number of years considered; he suggests that a selective death rate may explain the result.

The material here dealt with was collected in Middlesbrough from various sources and refers to females only. The standard selected was the number of blue-eyed persons in groups of different ages. They were reached in the following ways.

Infants through the Notification of Births Act.

School children through the Education Act (1908) and

Adults through the attendance of parents consequent on the above.

The groups, though not true random samples, are not selected with respect to the character under investigation, and hence should be suitable for analysis.

The results were as follows:

TABLE I.

Females only.

Age	No. examined	Blue eyes
At birth	1000	100 %
6th year	387	54 %
9th ,,	488	41 %
10th ,,	391	41 %
13th ,,	400	38 %
15th to 29th year	80	32 %
31st ,, 40th ,,	543	30 %
41st ,, 45th ,,	320	28 %
46th ,, 55th ,,	269	26 %

From these figures it is safe to conclude either that a tendency for the eye to darken exists through life, being most marked at birth and becoming less as time advances, or that death is strongly selective for this character. The former hypothesis is the more reasonable one. In later life a further alteration occurs which can be attributed to the loss of translucency of the stroma. It is obvious that eye-colour cannot be strictly regarded as a fixed character, so that some information as to age is necessary. In the adolescent period the change however is not marked.

There is of course no apparent reason why age of parent at birth should influence eye-colour, so that the result of an investigation of such a point must be *a priori* incapable of prediction.

The subject therefore under consideration resolves itself into the question of the existence rather than the nature of such a biological sequence.

For this purpose blue eyes were chosen and the number occurring in the various groups noted, all details as to shade or formation being ignored.

The data were collected in Middlesbrough and Barking through the opportunities afforded by the Medical Inspection of School Children. The first series are taken from Barking and consist of children in their 7th and 13th years, in the proportion of three to one. The mother's eye colour was also taken so as to correct for any tendency of a particular type of eye-colour being more fertile and hence occurring more frequently than would be expected in a true random sample.

The tables are as follows:

TABLE II.

All Children.

		Child.		
		Blue	Not blue	Totals
		88	55	143
Mother:	Blue	64	247	311
	Not blue	152	302	454

$r = \cdot 5966 \pm \cdot 0253.$

TABLE III.

Age of Parent at Birth and Child's Eye-colour.

Age of parent at birth	Child's eye-colour		
	Blue	Not blue	Totals
16—20	4	7	11
21—24	38	67	105
25—28	36	74	110
29—32	29	66	95
33—36	23	47	70
37—40	8	23	31
41—44	10	14	24
45—48	3	3	6
Totals	151	301	452

$r = \cdot 0961 \pm \cdot 0334.$

$\eta = \cdot 1031$ and if corrected, indeterminate[1].

TABLE IV.

Age of Parent at Birth and her own Eye-colour.

Age of parent at birth	Mother's eye-colour		
	Blue	Not blue	Totals
16—20	5	6	11
21—24	35	63	98
25—28	25	76	101
29—32	26	59	85
33—36	20	41	61
37—40	8	19	27
41—44	6	6	12
45—48	1	4	5
Totals	126	274	400

$r = \cdot 0034 \pm \cdot 0337.$

$\eta = \cdot 0611$ and if corrected, indeterminate.

[1] Pearson, K. (1912). A correction to be made to the Correlation Ratio. *Biometrika* VIII. 254.

The coefficients are:

Age of mother at birth of child (1) and child's eye-colour (2)

$$r_{12} = \cdot 096 \pm \cdot 033.$$

Age of mother 9 years after birth of child (1) and her own eye-colour (3)

$$r_{13} = \cdot 003 \pm \cdot 034.$$

Eye-colour of mother (3) and child (2)

$$r_{23} = \cdot 597 \pm \cdot 025.$$

Making eye-colour of mother (3) constant then:—

Age of mother at birth of child (2) and child's eye-colour (1)

$$_3r_{12} = \cdot 13 \pm \cdot 03.$$

This result suggests that children born of young mothers have a tendency to blue eyes, or that the change dependent on age is delayed. The values of the correlation ratios were found to be, mean of child's eye-colour for arrays of age at birth, $\eta = \cdot 103 \pm \cdot 033$, and if corrected becomes indeterminate; the same with means of mother's eye-colour $\eta = \cdot 061 \pm \cdot 034$, and is also indeterminate if corrected by Pearson's method.

To test the data for any bias in the method of collection, the eye-colour of the child was correlated with a different mother from its own. The data are as follows:

TABLE V.

	Random mother		
Child:	Blue	Not blue	Totals
Blue	49	91	140
Not blue	103	207	310
Totals	152	298	450

$$r = \cdot 0362 \pm \cdot 0302.$$

TABLE VI.

Age of Random Parent at Birth and Child's Eye-colour.

Age of parent at birth	Random child: Blue	Not blue	Totals
16—20	2	4	6
21—24	20	33	53
25—28	21	30	51
29—32	10	24	34
33—36	12	12	24
37—40	8	7	15
41—44	2	6	8
45—48	1	0	1
Totals	76	116	192

$$r = - \cdot 0306 \pm \cdot 0477$$

Age of random parent at birth (1) and child's eye-colour (2)

$$r_{12} = -\cdot 031 \pm \cdot 048.$$

Eye-colour random mother and child

$$r_{13} = \cdot 036 \pm \cdot 031.$$

Hence. Age of random parent at birth and child's eye-colour—parent constant

$${}_3r_{12} = -\cdot 03 \pm \cdot 05.$$

A result that suggests that the sample taken is not subject to any great error in collection.

In the second series of the data, collected in Middlesbrough, the age period was restricted to children of a particular age (8th to 9th year, *i.e.* born in year 1900). The sexes are dealt with separately and in each case the eye-colour of the mother is recorded, so as to remove any bias due to stock. The data are as follows:

TABLE VII.

Age of Mother at Birth of Child and her own Eye-colour.

Based on girls:	Mother's eye-colour		
Age of mother	Blue	Not blue	Totals
20 and under	9	24	33
21—25	38	78	116
26—30	42	100	142
31—35	26	81	107
36—40	23	51	74
41 and over	3	13	16
Totals	141	347	488

$r = \cdot 0171 \pm \cdot 0303.$

$\eta = \cdot 118 \pm \cdot 029$ and if corrected $= \cdot 0607.$

TABLE VIII.

Based on boys:	Mother's eye-colour		
Age of mother	Blue	Not blue	Totals
20 and under	7	15	22
21—25	35	71	106
26—30	36	97	133
31—35	21	64	85
36—40	13	43	56
41 and over	3	20	23
Totals	115	310	425

$r = \cdot 0494 \pm \cdot 0299.$

$\eta = \cdot 1592 \pm \cdot 0334$ and if corrected $= \cdot 1262.$

TABLE IX.

Age of Mother at Birth of Child and Child's Eye-colour (Girls).

Age of mother at birth	Child's eye-colour: Blue	Not blue	Totals
Under 20	12	21	33
21—25	56	60	116
26—30	58	84	142
31—35	41	66	107
36—40	30	44	74
41 and over	4	12	16
Totals	201	287	488

$r = \cdot 0355 \pm \cdot 0302$.
$\eta = \cdot 1289 \pm \cdot 0299$ and if corrected $= \cdot 0798$.

TABLE X.

Boys.

Age of mother at birth	Child's eye-colour: Blue	Not blue	Totals
20 and under	13	9	22
21—25	46	60	106
26—30	53	80	133
31—35	30	55	85
36—40	21	35	56
41 and over	5	18	23
Totals	168	257	425

$r = \cdot 0537 \pm \cdot 0301$.
$\eta = \cdot 2409 \pm \cdot 0324$ and if corrected $= \cdot 2151$.

TABLE XI.

Boys. 7—8 *years.*

Mother:	*Child.* Blue	Not blue	Totals
Blue	79	34	113
Not blue	88	223	311
Totals	167	257	424

$r = \cdot 5868 \pm \cdot 0216$.

TABLE XII.

Girls. 7—8 *years.*

Mother:	*Child.* Blue	Not blue	Totals
Blue	104	36	140
Not blue	103	240	343
Totals	207	276	483

$r = \cdot 6211 \pm \cdot 0193$.

From these figures for boys we have:

Age of mother at birth of child (1) and child's eye-colour (2)

$$r_{12} = \cdot 054 \pm \cdot 030.$$

Age of mother at birth of child (1) and her own eye-colour (3)

$$r_{13} = \cdot 049 \pm \cdot 03.$$

Child's and mother's eye-colour

$$r_{23} = \cdot 587 \pm \cdot 023.$$

Making mother's eye-colour constant

$$_3r_{12} = + \cdot 03 \pm \cdot 03.$$

The correlation ratios are:

Mean of eye-colour, child, for arrays of mother's age

$$\eta = \cdot 24 \pm \cdot 03 \text{ and if corrected} = \cdot 21.$$

Mean of eye-colour, mother, for arrays of her age

$$\eta = \cdot 16 \pm \cdot 03 \text{ and if corrected} = \cdot 13.$$

In the case of girls, we have:

Age of mother at birth of child (1) and child's eye-colour (2)

$$r_{12} = \cdot 036 \pm \cdot 030.$$

Age of mother at birth of child (1) and her own eye-colour (3)

$$r_{13} = \cdot 017 \pm \cdot 03.$$

Eye-colour, mother (3), eye-colour, child (2)

$$r_{23} = \cdot 621 \pm \cdot 019.$$

Making mother's eye-colour (3) constant

$$_3r_{12} = \cdot 04 \pm \cdot 03.$$

The correlation ratios for arrays of mother's age at birth and mean of child's eye-colour

$$\eta = \cdot 13 \pm \cdot 03 \text{ and if corrected} = \cdot 08.$$

For arrays of mother's age and her own eye-colour

$$\eta = \cdot 12 \pm \cdot 03 \text{ and if corrected} = \cdot 06.$$

The values found in the south (Barking) are not identical with those found in the north (Middlesbrough). In the first, a small significant positive correlation exists; in the second series the value is still positive for both sexes, but it is not significant. The differences may be dependent on the fact that making the mother's eye-colour constant does not completely remove the effects that may arise from the different reproductive habits that are to some extent associated with eye-colour.

Although these results are of interest and suggest that some biological difference may exist of the nature suggested, they do not justify any definite assertion.

If we now consider age with respect to age of grandmother at birth of mother, taking the eye-colour of mother and child (the actual material is the same as in the previous series) we have:

TABLE XIII.

Age of Grandmother at Birth. Eye-colour of Mother and Child.

	Mother's eye-colour		
	Blue	Not blue	Totals
Mother born at 20 years and under	35	97	132
„ „ 21—25	60	157	217
„ „ 26—30	74	161	235
„ „ 31—35	46	129	175
„ „ 36—40	26	75	101
„ „ 41 and over	15	38	53
Totals	256	657	913

$r = \cdot 002 \pm \cdot 021$. $\eta = \cdot 06$ and if corrected, indeterminate.

TABLE XIV.

	Child's eye-colour		
	Blue	Not blue	Totals
Mother born at 20 years and under	16	39	55
„ „ 21—25	73	149	222
„ „ 26—30	78	179	257
„ „ 31—35	47	145	192
„ „ 36—40	36	94	130
„ „ 41 and over	6	33	39
Totals	256	639	895

$r = \cdot 032 \pm \cdot 021$. $\eta = \cdot 123$ corrected. $\eta = \cdot 098$.

From these observations we find:

Age of grandmother at birth of mother, and eye-colour of child

$$r = \cdot 032 \pm \cdot 021.$$

Age of grandmother at birth of mother, and eye-colour of mother

$$r = \cdot 002 \pm \cdot 021.$$

The correlation ratios are:

Mean of child's eye-colour for arrays of grandmother's age

$$\eta = \cdot 123 \pm \cdot 02 \text{ and if corrected } = \cdot 098.$$

Mean of mother's eye-colour for arrays of grandmother's age

$$\eta = \cdot 061 \pm \cdot 021 \text{ and if corrected, indeterminate.}$$

From this series it would seem as if there was no significant association between age of parent at birth and the eye-colour of an adult, hence from the previous observations, which suggest some association, it is possible that the rate at which the eye-colour develops from its initial blueness to its subsequent tint is modified, the change being more rapid in the later born. The subject could be more easily investigated by observing the alteration of tint in the first year of life. On the whole, however, we must regard the result of this investigation as purely negative.

Stature or Growth.

The relationship of growth or stature to age at birth has received considerable attention from different observers, amongst whom may be mentioned Gini, Marro, Stamini, Fourman, Budin, Ribemont, Schartzel, Hecker, and collected series of observations are also given in Vierordt's *Anatomische Daten u. Tabellen*, and in Prinzing's *Handb. d. medizinischen Statistik.*

The major portion of the work done by these observers has concerned itself with the development of the human infant at birth. Beyond the crude analysis of the figures little attempt has been made to separate the numerous factors that must necessarily play a more or less prominent part in modifying the development of the offspring. Analysis of the work of these observers shows that the variations are statistically significant, but concordance is wanting.

Thus according to age at birth, the mean weight and length of the infant increases, as age of mother at birth advances, whilst in association with order, the first or earlier born have the advantage. The influence appears to vary, according to whether the observations do or do not include those more comfortably placed economically. It is possible that the differences can be explained by the fact that the earlier born come in an undue proportion from the smaller families and those of the better social grades.

It has been shown by Arkle and many others that social position is associated with a better physical development so that should order be made constant, the apparent effect of age becomes more marked.

Further it has been suggested that the length of the puerpural period increases as age advances, hence the infant must not be regarded as starting life at the time of birth. Gini, comparing the expected weight of an infant making the observed order constant, shows that there is no significant variation with respect to age at birth, but his method does

not allow for the physical development of the parents, and in so far as the earlier numbers are of a more heterogeneous type than the later, the process of calculation adopted cannot be said to have eliminated the disturbing factor of order.

In the following series of observations the age of both parents has been recorded. It was hoped that by making the age of mother constant for given age of father, that nutritive influences dependent on age of mother would be removed.

As will be seen no significant alteration is made in the final result. The material was collected in Middlesbrough under the Education Act, Administrative Provision, 1907.

TABLE XV.

Age of Father at Birth of Child and its Height.

Boys.

	Inches														
Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20—23	1	1	4	5	8	20	13	6	6	2	—	—	—	—	66
24—27	2	2	3	11	20	43	33	16	7	8	—	4	3	—	152
28—31	—	4	6	12	17	20	28	22	23	13	—	—	2	—	147
32—35	2	1	2	7	10	21	24	23	12	9	2	—	1	—	114
36—39	1	1	3	7	18	15	16	10	8	2	1	2	—	—	84
40—43	—	—	3	1	12	16	9	12	3	2	—	1	—	—	59
Over 43	—	1	2	2	3	6	9	4	4	1	1	—	—	—	33
Totals	6	10	23	45	88	141	132	93	63	37	4	7	6	—	655

$\sigma_{height} = 2{\cdot}0083$. $\sigma_{age} = 1{\cdot}2572$. $r = {\cdot}0213 \pm {\cdot}0261$. $\eta = {\cdot}0722 \pm {\cdot}022$.

TABLE XVI.

Age of Father at Birth of Child and its Height.

Girls.

	Inches														
Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20—23	—	3	2	8	7	11	21	9	5	—	—	—	—	—	66
24—27	—	5	10	10	12	29	19	14	14	6	5	1	—	—	125
28—31	4	1	1	6	23	19	41	18	5	7	1	—	—	—	126
32—35	2	1	5	11	12	27	26	14	9	2	2	—	—	—	111
36—39	1	2	5	6	11	15	13	10	8	4	—	—	2	—	77
40—43	1	1	—	6	6	18	12	9	3	2	1	2	—	—	61
Over 43	—	—	1	—	—	1	2	2	—	—	—	—	—	—	6
Totals	8	13	24	47	71	120	134	76	44	21	9	3	2	—	572

$\sigma_{height} = 2{\cdot}0054$. $\sigma_{age} = 1{\cdot}2743$. $r = {\cdot}0522 \pm {\cdot}0278$. $\eta_{height} = {\cdot}1263 \pm {\cdot}019$.

Boys and girls together, $r = {\cdot}0367 \pm {\cdot}0210$.

TABLE XVII.

Age of Mother at Birth of Child and its Height.

Girls.

	Inches														
Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	3	—	—	3	5	3	1	2	—	—	—	—	—	17
20—23	1	3	5	10	10	22	19	16	8	4	3	—	—	—	101
24—27	1	2	8	7	14	38	39	22	6	6	2	1	—	—	146
28—31	4	2	4	15	23	16	31	15	12	6	2	2	—	—	132
32—35	1	1	5	10	11	23	26	8	8	—	—	—	2	—	95
36—39	—	2	1	1	6	10	10	11	5	1	—	—	—	—	47
40—43	—	—	4	2	4	6	4	5	2	2	1	—	—	—	30
Over 43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals	7	13	27	45	71	120	132	78	43	19	8	3	2	—	568

$\sigma_{\text{height}} = 1{\cdot}9115$. $\sigma_{\text{age}} = 1{\cdot}2985$. $r = {\cdot}0675 \pm {\cdot}0287$. $\eta_{\text{height}} = {\cdot}1622 \pm {\cdot}015$.

TABLE XVIII.

Age of Mother at Birth of Child and its Height.

Boys.

	Inches														
Years	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Totals
16—19	—	—	2	3	4	8	2	—	6	1	—	—	—	—	26
20—23	2	3	4	9	17	26	25	17	7	7	—	—	—	—	117
24—27	1	4	4	15	13	41	29	26	22	14	2	—	1	1	173
28—31	—	1	6	6	19	23	34	19	8	12	—	5	2	—	135
32—35	2	1	—	6	17	16	20	17	10	1	1	1	1	—	93
36—39	—	1	5	4	13	20	16	4	7	—	—	1	—	—	71
40—43	—	—	2	2	6	6	6	11	4	3	1	—	—	—	41
Totals	5	10	23	45	89	140	132	94	64	38	4	7	4	1	656

$\sigma_{\text{height}} = 2{\cdot}0419$. $\sigma_{\text{age}} = 1{\cdot}3261$. $r = {\cdot}0266 \pm {\cdot}0259$. $\eta = {\cdot}1178 \pm {\cdot}018$.

Boys and girls together, $r = {\cdot}0471 \pm {\cdot}0211$.

TABLE XIX.

Age of Father and Mother at Birth of Child.

		Age of father in years					
		20 and under	21—25	26—30	31—35	36—40	41 and over	Totals
Age of mother in years	20 and under	7	26	7	4	—	—	44
	21—25	6	92	76	17	7	2	200
	26—30	—	18	123	92	17	6	256
	31—35	—	1	21	78	41	21	162
	36—40	—	—	4	4	61	51	120
	41 and over	—	—	—	1	6	43	50
	Totals	13	137	231	196	132	123	832

$\sigma_{\text{mother}} = 1{\cdot}2900$. $\sigma_{\text{father}} = 1{\cdot}3368$. $r = {\cdot}7858 \pm {\cdot}015$.

The coefficients evaluated are:

Age of father at birth of child (1) and its height (2)

$$r_{12} = \cdot 037 \pm \cdot 020.$$

Age of mother at birth of child (3) and its height (2)

$$r_{23} = \cdot 047 \pm \cdot 019.$$

Age of father (1) and age of mother (3)

$$r_{13} = \cdot 768 \pm \cdot 008.$$

Age of father and height of child, age of mother constant

$${}_3r_{12} = \cdot 001 \pm \cdot 021.$$

Age of mother and height of child, age of father constant

$${}_2r_{13} = \cdot 030 \pm \cdot 020.$$

η (mean of child's height for arrays of mother) $= \cdot 14 \pm \cdot 02$.

η (mean of child's height for arrays of father) $= \cdot 10 \pm \cdot 02$.

It would seem from these figures that there is no reason to believe that age *per se* has any effect on growth, but the environmental influence of the pre-natal period may have some effect. It is to be observed that the correlation ratios are significant, which suggests that the concomitant factors associated with age may be so arranged as to neutralise each other when viewed as a whole. It is necessary, therefore, to examine the matter in more detail before concluding that the effect of age at birth is negligible. This cannot be done without further material.

Age of Parent at Time of Birth and Intelligence.

The general literature on factors relative to intelligence is quite outside the scope of the present enquiry and that directly relevant to the subject matter seems to be limited to certain Italian writers, amongst whom are Lombroso, Orchansky, Marro, and Gini.

The conclusions arrived at by these writers are first that the influence of the mother's age predominates over that of the father's, and second that children born of younger parents seem to be more intelligent than those born of older parents. In the present paper the means of introduction were the same as in the previous sections, namely, through the Education Act of 1907. The standard of intelligence for parent and child was the ordinary scholastic test of proficiency; that is to say the age of the child and his or her standard; the mother's age on leaving

school and her standard; and her age when the child under consideration was born were the details taken. It is to be noted that the object is to consider all children as produced by a uniform mother. To neutralise the disturbing factors noted above, we should reduce the data to such as would be produced by a common ancestry, which has not been done; so that we cannot claim that the possibility of race variations has been neutralised by removing any association that may be dependent on peculiarities of the mother. Two series were collected, firstly, a group of those in their 6th year (born 1903) and those in their 13th year (born 1895). No separation was made as to the sex in calculating coefficients. The data are as follows:

TABLE XX.

Age of Mother at Birth of Child and her own standard on leaving school (child in 5th year).

Age at birth of child	Standard on leaving school: Standard VII		Other standards		
	Boys	Girls	Boys	Girls	Totals
20 and under	4	6	10	16	36
21—25	34	30	45	48	157
26—30	43	45	56	74	218
31—35	24	9	35	38	106
36—40	7	11	21	25	64
41 and over	2	3	6	1	12
Totals	114	104	173	202	593

$$r = -\cdot 2983 \pm \cdot 0251.$$

TABLE XXI.

Age of Mother at Birth of Child and her own standard on leaving school (child in 13th year).

Age at birth of child	Standard VII		Other standards		
	Boys	Girls	Boys	Girls	Totals
20 and under	1	7	5	6	19
21—25	23	23	24	30	100
26—30	20	23	21	27	91
31—35	12	14	19	16	61
36—40	6	6	5	4	21
41 and over	5	6	2	2	15
Totals	67	79	76	85	307

$$r = -\cdot 2609 \pm \cdot 0364.$$

TABLE XXII.

Age of Mother at Birth of Child and its standard in 5th year.

Age at birth of child	Class I Boys	Class I Girls	Other classes Boys	Other classes Girls	Totals
20 and under	9	20	3	3	35
21—25	58	65	28	20	171
26—30	88	81	30	32	231
31—35	47	46	19	15	127
36—40	20	34	12	8	74
41 and over	5	7	3	3	18
Totals	227	253	95	81	656

$r = \cdot 0525 \pm \cdot 0274.$

TABLE XXIII.

Age of Mother at Birth of Child and its standard in 13th year.

Age at birth of child	Standard of child: Standard VII Boys	Standard VII Girls	Other standards Boys	Other standards Girls	Totals
20 and under	1	6	4	6	17
21—25	14	17	29	37	97
26—30	12	14	22	30	78
31—35	4	4	22	20	50
36—40	2	1	5	5	13
41 and over	1	2	2	1	6
Totals	34	44	84	99	261

$r = \cdot 067 \pm \cdot 0387.$

TABLE XXIV.

Standard of Mother on leaving school, and class of Child in 5th year.

Standard of mother	Class of child: Class I Boys	Class I Girls	Other classes Boys	Other classes Girls	Totals
Standard VII	88	77	28	25	218
Other standards	122	148	61	46	377
Totals	210	225	89	71	595

$r = \cdot 534 \pm \cdot 0207.$

TABLE XXV.

Standard of Mother on leaving school, and standard of Child in 13th year.

Standard of mother	Standard of child: Standard VII — Boys and girls	Other standards — Boys and girls	Totals
Standard VII	43	34	77
Other standards	60	120	180
Totals	103	154	257

$r = \cdot 331 \pm \cdot 0354.$

TABLE XXVI.

Age of Mother when leaving school, and her standard (child in 5th year).

Age of mother	Standard VII	Other standards	Totals
14 years	149	114	263
13 ,,	45	144	189
12 ,,	25	85	110
11 ,,	3	31	34
10 ,,	—	8	8
9 ,,	—	2	2
Totals	222	384	606

$r = + \cdot 4975 \pm \cdot 0231.$

TABLE XXVII.

Age of Mother when leaving school, and her standard (child in 13th year).

Age of mother	Standard VII		Other standards		Totals
	Boys	Girls	Boys	Girls	
14 years	16	26	21	27	90
13 ,,	15	13	27	29	84
12 ,,	1	5	21	24	51
11 ,,	2	0	12	10	24
10 ,,	—	—	—	3	3
9 ,,	—	—	1	1	2
8 ,,	—	—	—	1	1
Totals	34	44	82	95	255

$r = + \cdot 4334 \pm \cdot 0327.$

TABLE XXVIII.

Age of Mother when leaving school, and standard of Child in 5th year.

Age of mother	Class I	Other classes	Totals
14 years	192	64	256
13 ,,	130	51	181
12 ,,	81	29	110
11 ,,	22	10	32
10 ,,	6	1	7
9 ,,	2	0	2
Totals	433	155	588

$r = + \cdot 0129 \pm \cdot 0275.$

TABLE XXIX.

Age of Mother when leaving school, and standard of Child in 13th year.

Age of mother	Standard VII	Other standards	Totals
14 years	26	49	75
13 "	31	39	70
12 "	10	34	44
11 "	5	12	17
10 "	2	—	2
9 "	—	1	1
8 "	1	—	1
Totals	75	135	210

$r = \cdot0184 \pm \cdot0477$.

TABLE XXX.

Age of Mother at Birth of Child and her age on leaving school (child in 5th year).

Age of mother at birth of child	Age of mother on leaving school						
	14 years	13 years	12 years	11 years	10 years	9 years	Totals
20 years and under	15	14	4	2	1	—	36
21—25	71	49	26	7	5	1	159
26—30	89	71	47	10	1	—	218
31—35	45	24	27	9	—	1	106
36—40	33	27	4	4	—	—	68
41 and over	8	—	2	2	—	—	12
Totals	261	185	110	34	7	2	599

$r = -\cdot031 \pm \cdot027$.

TABLE XXXI.

Age of Mother at Birth of Child and her age on leaving school (child in 13th year).

Age of mother at birth of child	Age of mother on leaving school						
	14 years	13 years	12 years	11 years	10 years	9 years	Totals
20 years and under	4	5	4	2	—	—	15
21—25	30	34	19	12	1	—	96
26—30	31	22	15	5	2	—	75
31—35	17	18	10	3	—	1	49
36—40	3	5	1	3	—	—	12
41 and over	4	2	1	—	—	—	7
Totals	89	86	50	25	3	1	254

$r = -\cdot082 \pm \cdot038$.

The values found for children in the 5th and 13th years were as follows:

		Children in	
		5th year	13th year
Age of mother on leaving school (1) and her own standard (2)	r_{12} =	·498 ± ·023	·433 ± ·033
Age of mother on leaving school (1) and class of child in 5th and 13th years (3)	r_{13} =	·013 ± ·028	·018 ± ·048
Age of mother at birth of child (4) and its class in 5th or 13th year (3)	r_{13} =	·053 ± ·027	·067 ± ·039
Age of mother at birth of child (4) and her own standard (2)	r_{42} =	− ·298 ± ·025	− ·261 ± ·035
Standard of mother on leaving school (2) and class of child in 5th or 13th year (3) ...	r_{23} =	·534 ± ·021	·331 ± ·035
Age of mother on leaving school (1) and age of mother at birth of child (4)	r_{14} =	− ·031 ± ·027	− ·082 ± ·038

The two series show a fair agreement, although the associations revealed are small. The following inferences may be drawn.

I. That there is a significant association between the age at which the mother left school and the standard she had attained. With respect to her child's intellectual standard, it is not significant.

II. That the age of the mother when the child was born is associated with her own intellectual grade, the sign being negative; that is, those who reproduce in late life are of a lower intellectual grade. With respect to the child's standard, the association is positive, but very small.

III. That the intellectual standards taken are highly correlated in mother and child, and on that account are probably a fair test of the point under investigation. It is worthy of note, that the association between mothers and children is greater in the fifth year than in the thirteenth year. This is due either to the fact that the latter group is selected from children of more careful parents than the former, or that children are promoted according to age rather than ability.

The intellectual grading of children in our elementary schools has been investigated statistically by Pearson and Jones (*Biometrika*, Vol. VII. p. 542). Mrs Frances Wood has also dealt with this subject in the Barking area, and she has kindly allowed me to use her results—as yet unpublished. The two investigations placed side by side are:

	Barking, North St School	Pearson and Jones
r, age and standard ... ... =	+ ·73	+ ·94
r, intelligence and standard ... ... =	+ ·40	− ·06
r, age and intelligence ... ... =	− ·215	− ·183
r, intelligence and standard with age constant =	+ ·83	

The value obtained for $_{a}r_{c}$ ($= \cdot 83$) points to a very definite system of promotion according to ability. Further $r_{,} = - \cdot 40$ would suggest that the more able children tended to pass into the higher classes, irrespective of age, for the correlation between age and intelligence is $- \cdot 215$.

The trend of these results rather suggests that promotion depends upon the personnel of the school, and on the accommodation. The former hardly needs comment. It is obvious that in a series of classrooms to hold 60 children and every classroom full, promotion according to age irrespective of intelligence must occur. On the other hand if classrooms accommodate 60 children and the average size of class is 40, grading according to intellect becomes possible. In the Middlesbrough area in which the data of this paper were collected, the overcrowding of the schools was considerable.

IV. The fourth value is what would be expected: that is, mothers who reproduce late, tend to have left school early.

We can now proceed to remove the disturbing factors, but it must be remembered that, with a mother of constant intelligence, racial effects have not been removed, but only reduced.

Making age at which the mother left school, and her standard constant (that is, considering the problem as if all children came from mothers of constant intelligence), then the correlations between age of mother at birth of offspring and its intellectual grade are, for children in the 5th year $r = \cdot 32 \pm \cdot 04$ and for children in the 13th year

$$r = \cdot 17 \pm \cdot 03.$$

It is seen that a crude correlation of $\cdot 05$ is increased to approximately $\cdot 25$ when the character examined is made constant for the female parent. The same point has been noted before, namely, that the effect of parental age can only be measured when the various compensating factors are removed.

The remaining partial coefficients are given in the following table.

2nd order

	Child in 5th year	Child in 13th year
$_{34}r_{12}$	$\cdot 59 \pm \cdot 02$	$\cdot 45 \pm \cdot 02$
$_{24}r_{13}$	$- \cdot 44 \pm \cdot 03$	$- \cdot 30 \pm \cdot 03$
$_{23}r_{14}$	$\cdot 24 \pm \cdot 04$	$\cdot 06 \pm \cdot 03$
$_{14}r_{23}$	$- \cdot 38 \pm \cdot 03$	$- \cdot 14 \pm \cdot 03$
$_{13}r_{24}$	$\cdot 32 \pm \cdot 04$	$\cdot 17 \pm \cdot 03$
$_{12}r_{34}$	$\cdot 65 \pm \cdot 02$	$\cdot 35 \pm \cdot 02$

It is to be noted that the correlation between age of mother at birth and age of mother on leaving school, with other factors constant, is

higher for children in the 5th year than in the 13th. This is an expected result, as reproduction in late life means a larger family, and the withdrawal from school at the earliest opportunity of children approaching the 13th year. It is obvious that data of this kind are subject to many curious influences which are extremely difficult to remove and, in fact, cannot be completely neutralised. It must therefore be with extreme hesitation that any inferences are drawn from the data collected in this way.

GENERAL CONCLUSIONS.

I shall now briefly summarise the conclusions I feel justified in drawing from the evidence submitted in the series of communications, of which the present is the fourth, reserving a full discussion for another occasion.

It appears, then, that as the age of the parent at the birth of the child increases, (*a*) the average length of life of the offspring decreases; (*b*) the fertility of the offspring increases; (*c*) the offspring react less characteristically to zymotic infections; (*d*) the proportion of males born increases; (*e*) the rate of change from congenital to ultimate type of eye-pigmentation of the offspring increases; (*f*) the intellectual grade of the offspring as defined by a scholastic standard rises.

Each of these conclusions depends upon the discovery of statistical constants significant with regard to an estimated error of sampling, but in no single case is the absolute intensity of the relation between the variables measured large, with two exceptions each of which is suspect, owing to the necessary ambiguity of the measures chosen by, or rather imposed upon, the investigator by the nature of the case.

In effect, the evidence that the populations of children born to parents of greater or less age are differentiated is inconclusive in any particular case taken by itself, but when we remember that a large variety of disparate attributes has been studied, that the particular fallacies inherent in each special investigation are likewise different, and that nevertheless all the results tend in the same direction, it is, I think, legitimate to infer that the populations are really differentiated.

Now we can suppose that the age of the parent may affect the characters of the offspring in the following ways.

Directly. In that (*a*) the primordial germ cells formed in late life partake of the senescence characterising the somatic cells, (*b*) the germ cell up to fertilisation is unaffected by senile somatic changes but thereafter suffers from nutritive effects dependent upon the soma.

Indirectly. In that the parental populations are differentiated, that those persons who bear or procreate children late in life are of a different type from others.

With respect to the second group of possibilities, we have already seen that in practice there is an economic differentiation which seriously complicates our analysis, for the more wealthy and educated classes marry later and cease to bear children earlier than the poorer members of the community. A good deal of statistical work in these papers has been undertaken with the object of eliminating this indirect factor, how far successfully is for the reader to judge, but the author recognises that the attempted neutralisation, particularly in the case of intelligence dealt with in the last part of the present paper, may well be deemed partly successful only.

Reverting to the direct effects, it may be said that although the Weismannian tradition, that of the inviolability of the germ plasm, has been notably weakened by the impact of hostile researches, yet even now the possibilities scheduled under (*b*) will seem to most of us the more inviting.

We may, however, assert that the (*b*) factors cannot suffice to account for all the facts. Thus, in all the quantitatively measurable characters studied, it has been found that the children of the younger or elder parents show changes in variability that cannot be attributed to maternal influence, which, of course, (*b*) covers completely. The data grouped in this way (taken from the peerage and referring to eldest born sons) are as follows:

Standard deviation of age of son at death, when born

before and including 31st year	32nd year or later
7·0865 ± ·1647	7·8033 ± ·1909

Diff. ·7168 ± ·2521. Ratio 2·84.

Age of father at death who reproduced

before and including 31st year	32nd year or later
7·0378 ± ·1327	6·3474 ± ·1514

Data with respect to the female parent are not forthcoming in the case of the peerage, but the variability in the number of children dying in a working class family is:

Standard deviation of number of children dying of mothers, born of grandparent

less than 30 years of age	more than 30 years of age
1·828 ± ·0344	1·888 ± ·0456

The difference is not significant and the suggestion is that the foregoing results can hardly be explained by reference to the female parent.

It may be of interest to examine the other characters dealt with from this point of view, taking growth, and dividing our data at the 30th year.

Standard deviation of height of mothers who reproduced a child

before and including 30th year	after 30th year
2·3550 ± ·0479	2·1326 ± ·0540

Height of children in 9th year—reproduced

before and including 30th year	after 30th year
2·3428 ± ·0479	2·4436 ± ·0618

Diff. ·1008 ± ·078. Ratio 1·4.

It is worthy of remark that the increased variability of the later born is itself a differentiation and a rather unexpected one. By the nature of the case the parents who reproduce late in life are a selection, a certain portion of the whole frequency of the parents being excluded. This kind of selection will tend to diminish the variability of the parental generation and to a less extent that of the filial generation. The stringency of the selection is not so intense nor the force of heredity so great in the case of the variables studied that we should expect any great reduction of variability; assuredly, however, we should not anticipate an increase which nevertheless we find.

There is however one apparent exception to the above. It is as follows (peerage data):

Standard deviation of number of offspring of son born

before 32nd year of father	after 32nd year of father
3·7127 ± ·0762	3·4561 ± ·073

Diff. ·256 ± ·091. Ratio 2·4.

This is contrary to what would have been expected as the increased variability of the life period of later born sons should lead to an increase in variability in the number of offspring produced. With respect to the female parent (working class data) the same trend is shown but the difference is not significant.

Standard deviation of number of children of daughter born

before 30th year of mother	after 30th year of mother
2·878 ± ·0499	2·7503 ± ·0572

Diff. ·12 + ·07.

This exception may be a special adaptation and not a true exception to what seems to be a general rule.

It agrees however with the following series in one respect, namely that an increase of the standard deviation is not accompanied by a corresponding change in the mean[1]. In these cases the characters of the parental generation could not be obtained.

Standard deviation of the age of attack of scarlet fever, of persons born

	before 30th year of mother	after 30th year of mother
	3·7072 ± ·0559	4·4227 ± ·0793
Mean	7·27 ± ·03 years	7·29 ± ·04 years

Standard deviation of the age of attack of diphtheria, of persons born

	before 30th year of mother	after 30th year of mother
	3·5635 ± ·0833	3·8769 ± ·1117
Mean	6·07 ± ·04 years	5·92 ± ·05 years

Standard deviation of the age of attack of small pox, of persons born

before 30th year of mother	after 30th year of mother
2·6977 ± ·1103	2·8933 ± ·1255

With the steady improvement of the method of compiling and presenting anthropometric data consequent upon the enlargement of the scope of public health administration, we may look to the provision of material fit to provide a basis for a more complete and searching investigation than I have found possible. In the meantime these results will be of service to those who wish to inquire into a fascinating and, it may be, sociologically important subject.

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ON THE STATISTICAL INTERPRETATION OF SOME BACTERIOLOGICAL METHODS EMPLOYED IN WATER ANALYSIS.

By M. GREENWOOD, Junr. and G. UDNY YULE.
(*Lister Institute of Preventive Medicine.*)

(With 3 Charts.)

We were recently consulted by an officer serving on the Western Front as to the significance attaching to ordinary bacteriological methods of gauging the potability of waters. He wished to know what was the probability that a given water supply did not contain more than a certain proportion of bacteria in the unit volume, it having been found that particular samples tested showed no growth while, perhaps, larger samples had done so, or that so many out of a series of samples of the same size had given positive results. Having obtained what seemed to us a reasonable solution of the particular problem proposed, we thought that the results might interest other officers and bacteriologists who have to do similar work. A survey of the criteria actually used by bacteriologists when they form an opinion as to the purity of waters seems to us to emphasise the need for some discussion.

So far as we can learn, the standard method is to find the minimum quantity of water from which a culture of the organism in question is obtained, usually at a single trial. It is also generally held that a useful indicator of pollution is furnished by the *B. coli* group; the difference between such highly refined techniques as that of the Metropolitan Water Board's experts and the rapid Field Service method introduced by Lieut.-Colonel P. S. Lelean is that the former envisage a carefully defined and limited group of organisms, while the latter merely determines the presence of such as ferment lactose, when grown in MacConkey's bile salt broth, within 24 hours. It will be remembered that Houston

has expressed positive results in terms of the numbers of tubes containing "flaginacs," *i.e.* organisms which:

(1) give greenish fluorescence (fl) in neutral-red broth,
(2) acid and gas (ag) in lactose-peptone,
(3) indol (in) in broth,
(4) acidity (ac) and clotting of litmus-milk.

Whether this rigorous examination really excludes many organisms which, although passing the lactose fermentation test, ought not to be admitted into the *B. coli* fold, is a question we are incompetent to answer.

With respect to standards, Dr Savage, who has worked in the Metropolitan Water Board's laboratory, writes that any deep well or spring water which contains *B. coli* (rigidly defined by such methods as that above detailed) in a sample of 100 c.c. or less should be regarded with great suspicion. In the case of surface supplies and shallow wells, he writes: "If no *B. coli* are present in 50 c.c., the water may probably be safely passed as satisfactory, as far as conditions actually present are concerned." "For rivers used as sources of drinking water, without artificial purification, similar standards are applicable[1]."

Colonel Lelean's standard is not defined. In practice he appears to have used 7 tubes containing respectively 20, 15, 10, 5, 2, 1 and $\frac{1}{2}$ c.c. of water to be tested[2]. The total volume of water so used is said to have been 50 c.c. (in reality, as it appears, $53\frac{1}{2}$ c.c.) and the tests "giving all negatives were recorded as having fractors in 75 c.c. instead of 50 c.c., while the all-positive results were given a value of fractors in $\frac{1}{4}$ c.c. instead of $\frac{1}{2}$ c.c." This novel statistical approximation does not, however, seem to have been uniformly employed by Colonel Lelean, for the number 50, and also the number 25, is frequently to be found in the column of his tables headed "Minimal number of c.c. containing lactose fractors." As no single tube of his series contained either 50 or 25 c.c. it is not obvious how these results were reached.

Nor is Colonel Lelean the only writer from the perusal of whose lucubrations the student may rise with some sense of bewilderment. Professor Hewlett[3] states that for the examination of an ordinary

[1] Savage, *The Bacteriological Examination of Water Supplies*, pp. 185–6. London, 1906.

[2] Bacteriological Examination of Waters in the Field. *Journ. Royal Army Medical Corps.* Sept. 1914.

[3] *Manual of Bacteriology*, 5th Edition. London, 1914.

drinking water he usually employs five tubes with 1 c.c. of the water in each, two tubes (double strength) with 10 c.c. in each, and one tube (double strength) with 25 c.c." (p. 584). Then later he remarks (p. 587) "The detection and enumeration of *B. coli* are regarded by all as perhaps the most important part of water examination. The number of *B. coli* is estimated from the amounts of water that have been added to the tubes of media, which, however, assumes that the organism is regularly distributed throughout the sample, and this must so far as possible be ensured by thorough mixing. The results generally come out fairly concordantly, though irregularities exceptionally occur which can only be obviated by making duplicate sets of cultures. It is better to state the result as "*B. coli* present in ... c.c. of water" rather than to say that so many *B. coli* are present, though as a matter of fact the latter statement is approximately correct. Adopting the writer's method for *B. coli* (p. 584), if *none* of the tubes contains *B. coli*, we say that "*B. coli* is absent from 50 c.c."; if the 25 c.c. tube contains *B. coli*, but not the remainder, "*B. coli* is present in 25 c.c. but not in less, and so on."

"If nothing is known about the water, the following standards may be adopted:

(*a*) *Waters of good quality.* *B. coli* absent in 50 c.c. of the water.

(*b*) *Waters of medium quality.* *B. coli* present in 50 c.c. but absent in 25 c.c.

(*c*) *Waters of poor quality.* *B. coli* present in 50 c.c. and 25 c.c., but absent in 10 c.c.

(*d*) *Waters of suspicious quality.* *B. coli* present in 50 c.c., 25 c.c. and 10 c.c., but absent in 1 c.c.

(*e*) *Waters unfit for drinking.* *B. coli* present in 1 c.c. or less."

In following paragraphs there is some qualification of the standards here laid down, it being pointed out that in upland surface waters a high degree of contamination may only be due to pollution by the excreta of animals and therefore not dangerous, while in the case of spring or deep well water *B. coli* should be absent from at least 50 c.c.

In the passage cited from p. 587 it is not explained how "the number of *B. coli* is estimated from the amounts of water that have been added to the tubes of media" even on the assumption of regular distribution, nor how any amount of stirring can ensure regular results, any more than stirring up the tickets in a bag containing equal numbers of black

and white cards can ensure that one will always draw five black and five white out of ten. It is not clear how duplicate sets of cultures can obviate irregularities, when two or more sets may give different results. The student will agree that when he fails to get a positive in any one of the author's series of tubes "*B. coli* is absent from 50 c.c." But if the 25 c.c. tube gives a positive but none of the remainder, he may legitimately object to the statement "*B. coli* is present in 25 c.c. but not in less," seeing that what has really been found is that *B. coli* is present in one lot of 25 c.c. but not in another—the lot made up of five 1 c.c. tubes and two of 10 c.c. The concluding words of the paragraph "and so on" also open up a vista of possible questions. If one of the 10 c.c. tubes gives a positive and the other a negative, how is the result to be stated? If one, or two, or more of the 1 c.c. tubes give positives, but not the remainder, what conclusion is to be drawn? Passing then to the standards laid down under heads (*a*)—(*e*), how is the student to determine standard (*b*) from the author's series of tubes? If *B. coli* is "present in 50 c.c." in his series, it is most likely to be present in the 25 c.c. tube and is therefore not absent from 25 c.c.: if present in the 25 c.c. tube, it is also very probably present in one of the others which total to 25 c.c. and this makes the case worse. Standard (*b*) cannot therefore be determined from the given series of tubes. Standard (*c*) also fails: if none of the 10 c.c. or 1 c.c. tubes give a positive, the actual result is, as already pointed out, "*B. coli* present in one sample of 25 c.c. but not in another." If either of the 10 c.c. tubes give a positive, one cannot state that *B. coli* is absent from 10 c.c. Precisely similar criticisms apply to (*d*). If *B. coli* is absent from all the 1 c.c. tubes, it is absent from 5 c.c., not from 1 c.c. If any one of the 1 c.c. tubes give a positive, it is not "absent from 1 c.c."

The train of reasoning followed in the treatise of Colonel Beveridge and Major Wanhill is somewhat different. These authors employed the usual MacConkey medium and they classified waters in practically the same way as Savage, *e.g.* they held that the absence of *B. coli* from 100 c.c. indicated a very pure water, while presence in 100 but absence from 50 c.c. "is an indication of a good water, which has been polluted in some way, by animal or by human excreta, but in such small amount, or at such a distant period that there would not be much danger attaching to the use of such water for a town supply, if filtered" (p. 152). They further observed that "For temporary camps, if inspection could reveal the presence of no polluting agency and it could be surmised that the excreta of sheep or cattle were the cause, the presence of *B. coli* in

10 c.c. or less might be allowed, this number being often found in moorland streams, where human contamination is unlikely[1]."

Beveridge and Wanhill also provide an arithmetical illustration of the method of determining the density of bacilli in the source from an examination of samples each of the same volume. They say that if ten tubes each inoculated with 1 c.c. of water yielded three positive and seven negative results the sample may be considered to contain *B. coli* in every 3 c.c. of water, adding: "this is a rough estimate and is not mathematically accurate." We may observe that it was this somewhat delphic utterance which induced an officer on water duty to submit to us the problem which was the starting point of the present investigation.

Before detailing our investigation, we may emphasise certain considerations which were, no doubt, present in the minds of the various authors cited but seemed to them too trivial to state.

The fact that a given volume of water tested contains no bacilli, or none which will grow, does not prove that the source of supply is sterile, the point is merely that the greater the volume tested with negative results the smaller is likely to be the population of organisms existing in the supply; none of the writers has attempted to provide a scale of bacterial densities corresponding to the increase of the minimum quantity of water found sterile on examination. We think, indeed, that the tenor of the passages cited creates a presumption that the authors' criterion really is that sources *shown by other methods or found from practical experience* to be safe or to be unsafe have *usually* been found to give sterile readings when samples of the assigned size have been tested. This would explain, for instance, the lower standard adopted in the case of moorland waters. This is undoubtedly a reasonable attitude of mind enough, but it is necessary to remark that the process is not wholly satisfactory, since two observers both testing the same source on, say, the basis of a sample of 100 c.c. might obtain the one a positive, the other a negative result, so that the one would reject and the other pass the supply. Further, no criterion is provided of the increase in accuracy of prediction attained when two, three or more samples of 100 c.c. all give sterile readings.

The object of this paper is to provide such criteria or at least to indicate the method by which they may be obtained in any given case. The actual technique employed is so different in detail in different

[1] Beveridge and Wanhill, *The Sanitary Officer's Handbook of Practical Hygiene*, 2nd Edition. London, 1912.

cases (thus the Metropolitan Water Board use tubes with volumes in decreasing geometrical progression, 100 c.c., 10 c.c., 1 c.c., etc., while Colonel Lelean had tubes of diminishing volume but not diminishing uniformly), that it is not possible to provide a table of standards which will be of use to all observers. Such tables can be drawn up, with the help of general formulae obtained, but, as the arithmetic if simple is laborious, we have confined ourselves to the provision of a few illustrative examples.

SECTION I. PRELIMINARY PROPOSITIONS.

If in the water from which samples of, say, 1 c.c. each are drawn there exist B bacilli in all in a total volume of W c.c. of water, then, the distribution of bacilli being assumed to be random, the probable numbers of c.c. with 0, 1, 2, 3,... bacilli in each are given by the binomial expansion of

$$\left(\frac{W-1}{W}+\frac{1}{W}\right)^B \qquad \text{.............................(1).}$$

Since B and W are both very large indeed, (1) becomes by a well-known transformation originally given by Poisson[1]:

$$e^{-\lambda}\left(1+\lambda+\frac{\lambda^2}{2!}+\frac{\lambda^3}{3!}\cdots\cdots\right) \qquad \text{.....................(2)}$$

where $\lambda=\frac{B}{W}$. The problem then reduces itself to that of determining the appropriate value of λ and the probable reliability of its determination.

SECTION II. CASE OF A SINGLE TEST.

We first consider the case of a sample of N c.c. having been taken and found sterile.

The chance of this happening for a given value of λ, is, by (2), $e^{-N\lambda}$.

Now it is reasonable to assume that all values of λ from 0 to some upper limit w are *a priori* equally probable (by analogy with Bayes' postulate) so that the chance of λ being within the range $\lambda \pm \frac{1}{2}d\lambda$ is $d\lambda/w$ and the chance that, λ being within the range, the event happens is $e^{-N\lambda}$, so that the complete chance of λ not exceeding some assigned value κ is:

$$P=\frac{\int_0^\kappa e^{-N\lambda}\,.\,d\lambda}{\int_0^w e^{-N\lambda}\,.\,d\lambda}=\frac{1-e^{-N\kappa}}{1-e^{-Nw}}.$$

[1] *Recherches sur la Probabilité d. Jugements, etc.*, p. 190 and p. 206.

e^{-Nw} diminishes very rapidly as w increases and we may put $w = \infty$ so that the required chance is

$$P = 1 - e^{-N\kappa} \quad \text{.............................(3).}$$

Consequently if we desire to assign an upper limit to the probable density of bacilli in a source of supply a sample of N c.c. from which has proved sterile we have the following simple method. Suppose we take as our standard the limit corresponding to odds of 99 to 1.

Then, from (3)

$$e^{-N\kappa} = \cdot 01,$$

and the odds are 99 to 1 that κ is not greater than

$$-\frac{\log \cdot 01}{N \log e}.$$

Looking at the matter from the standpoint of the frequency distribution, we may say that the frequency distribution of bacilli per c.c. in waters which give a negative result on testing N c.c. is

$$y = N \, . \, e^{-N\lambda}.$$

The maximum is at zero and the curve tails off rapidly towards the higher densities. The actual curve for $N = 100$ is shewn in Fig. 1.

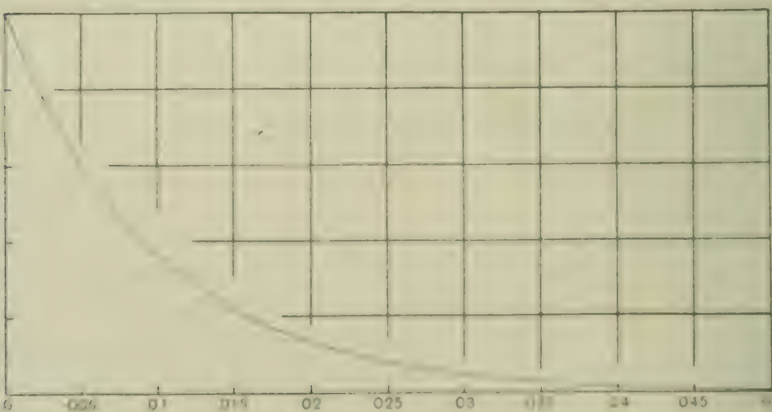

Fig. 1. Frequency distribution of bacterial densities when 100 c.c. give a negative result. The area of the curve is 10 squares.

SECTION III. CASE OF TWO TESTS.

We now take the instance of two tubes having been used with respectively n and m c.c. of water in each. Should both prove negative, we reach the previous result putting $n + m = N$. If the n c.c. tube is negative and the m c.c. tube positive then from (2) by similar reasoning we have for the chance that λ does not exceed κ:

$$P = \frac{\int_0^\kappa e^{-\lambda n}(1 - e^{-\lambda m})\, d\lambda}{\int_0^w e^{-\lambda n}(1 - e^{-\lambda m})\, d\lambda},$$

putting w = infinity, as before, we have

$$P = 1 - \left(\frac{n+m}{m} e^{-n\kappa} - \frac{n}{m} e^{-(n+m)\kappa}\right) \quad \text{...............(4).}$$

Taking again some particular value of odds, such as 99 to 1, (4) enables us to find a value of κ.

It is to be noted that the method breaks down if *both* samples give positive results for

$$\frac{\int_0^\kappa (1 - e^{-n\lambda})(1 - e^{-m\lambda})\, d\lambda}{\int_0^w (1 - e^{-n\lambda})(1 - e^{-m\lambda})\, d\lambda},$$

vanishes for $w = \infty$, so that no upper limit is assignable to λ.

The problem of this section may also be studied from the point of view of curve fitting. We have, writing $m + n = N$,

$$y = \frac{Nn}{m} e^{-\lambda n}(1 - e^{-\lambda m}) \quad \text{........................(5),}$$

for the distribution of λ, since the total area is

$$\int_0^\infty (1 - e^{-\lambda n})(1 - e^{-\lambda m})\, d\lambda = \frac{m}{Nn}.$$

The curve extends from 0 to ∞, and differentiating (5) with respect to λ and equating to zero we find for the mode

$$\lambda = \log \frac{N}{n} \cdot \frac{1}{m \log e} \quad \text{..........................(6).}$$

Now

$$\int_0^x x^n e^{-x\kappa} \,.\, dx = \frac{n}{\kappa}\int_0^x x^{n-1} \,.\, e^{-x\kappa} \,.\, dx = \ldots = \frac{n\,!}{\kappa^{n+1}}$$

(integrating successively by parts and noticing that the first part

vanishes), so that the successive moments of (5) are determinate. We have:

$$\text{mean} = \frac{N+n}{nN} \quad \text{.............................(7).}$$

$$\mu_2 = \frac{N^2+n^2}{n^2 \cdot N^2} \quad \text{.............................(8).}$$

$$\mu_3 = \frac{2\,(N^3+n^3)}{N^3 n^3} \quad \text{.........................(9).}$$

This curve therefore has positive skewness for all finite values of n, *i.e.* the long tail of the distribution extends to high values of λ.

Again if we write λ_1 for the modal value found in (6), the chance that the true bacterial density does not exceed the modal value is:

$$1 - \frac{Nn}{m}\int_{\lambda_1}^{\infty} (e^{-\lambda n} - e^{-\lambda N})\, d\lambda \quad \text{..................(9 a).}$$

integrating and putting λ_1 equal to (6), (9 a) becomes:

$$1 - \frac{1}{mN}\left(\frac{n}{N}\right)^{\frac{n}{m}} (N^2 - n^2) \quad \text{.....................(10).}$$

If $m = \rho n$, $N = (\rho + 1)\,n$, (10) becomes:

$$1 - \frac{1}{\rho\,(\rho+1)\,n^2}\left\{\frac{1}{(\rho+1)}\right\}^{\frac{1}{\rho}} \{(\rho+1)^2 n^2 - n^2\} = 1 - \frac{\rho+2}{(\rho+1)^{\frac{\rho+1}{\rho}}} \text{...(11).}$$

If ρ becomes very large the fraction approximates to unity and most of the area lies beyond the mode. This is reasonable, for the information that a second sample of infinite size gave a positive result adds nothing to our knowledge and (4) reduces to the case of the last section, for which, as λ cannot be negative, the mode is at $\lambda = 0$.

As an illustration of the forms that frequency distributions may take, the curves have been calculated (1) for the case in which 100 c.c. gives a positive result and 50 c.c. a negative, (2) for the reciprocal case in which 100 c.c. gives a negative but 50 c.c. a positive—an inconsistent but perfectly possible result. The two curves are shewn together in Fig. 2. Both distributions are very skew. The respective modes are $\lambda_1 = 0{\cdot}011$ and $\lambda_1 = 0{\cdot}0081$.

The question naturally arises, what is the probability of an inconsistence such as that assumed in the second illustration? If n c.c. have given a negative result what is the probability that a subsequent sample of m c.c. from the same water will also give a negative, or on the other

hand a positive? If n c.c. have given a negative, the probability that λ lies within the limits $\kappa \pm \frac{1}{2}d\kappa$ and that a second sample of m c.c. will then give a negative is $ne^{-N\kappa}\,d\kappa$, where $N = m + n$ as above. For

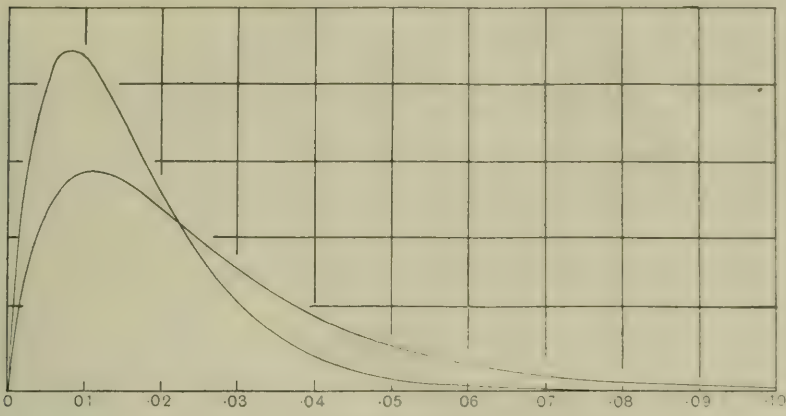

Fig. 2. Frequency distributions of bacterial densities for (1) 100 c.c. +, 50 c.c. −, the curve with the lower maximum, (2) 100 c.c. −, 50 c.c. +, the curve with the higher maximum. The area of each curve is 10 squares.

all values of λ the required probability is therefore the integral of this from 0 to ∞ or:

$$\left.\begin{array}{r}\text{chance that } n \text{ c.c. having given a negative, a second}\\ \text{sample of } m \text{ c.c. will also give a negative}\end{array}\right\} = \frac{n}{N}.$$

If $n = 100$, $m = 50$, the chance is $\frac{2}{3}$ or the chance of an inconsistence no less than $\frac{1}{3}$. If $m = n$, the chance is $\frac{1}{2}$. The results emphasise our previous remarks on the necessity of remembering that all results are subject to considerable fluctuations of sampling.

SECTION IV. CASE OF SEVERAL TESTS.

We first examine the case of N tubes, each containing the *same* quantity of water, which is taken as unity, n of the tubes being sterile while m give positive results.

If follows directly from (2) that the most probable value of λ is given by

$$\frac{N-m}{N} = e^{-\lambda} \text{ or } \lambda = \frac{1}{\log e}\log\frac{N}{N-m} = 2{\cdot}302585 \log\frac{N}{N-m} \quad \text{...(12).}$$

The value so obtained is, however, subject to wide fluctuations of sampling as we shall now show.

Calling $\frac{N-m}{N} = x$, we have

$$\sigma_x^2 = \frac{1}{N} \cdot \frac{N-m}{N} \cdot \frac{m}{N}.$$

If $$z = \log \frac{1}{x}, \quad dz = -\frac{1}{x} dx, \quad (dz)^2 = \frac{1}{x^2} (dx)^2,$$

and consequently

$$\sigma_z^2 = \frac{1}{x^2} \sigma_x^2 = \frac{m}{N(N-m)}.$$

And finally the "probable error" of λ determined from (12) is:

$$1{\cdot}553068 \sqrt{\frac{m}{Nn}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13).$$

As will be seen from the probable errors calculated as illustrations in the columns headed 4 and 10 of Table I the fluctuations are such that it seems better to resort to the method adopted in the previous sections.

TABLE I.

Probable number of bacilli per 1000 c.c. for a given number of blanks out of a given number of test tubes (1 c.c. in test). If 10 c.c. are placed in each tube instead of 1, divide all figures by 10.

No. of blanks	Out of								
	2	3	4	5	6	7	8	9	10
1	693	1098	1386	1609	1792	1946	2079	2197	2303
			± 1345						± 1473
2	—	405	693	916	1098	1253	1386	1504	1609
			± 777						± 982
3	—	—	287	510	693	847	981	1098	1204
			± 448						± 750
4	—	—	—	223	405	560	693	811	916
									± 602
5	—	—	—	—	182	336	470	588	693
									± 491
6	—	—	—	—	—	154	287	405	510
									± 401
7	—	—	—	—	—	—	134	251	357
									± 322
8	—	—	—	—	—	—	—	118	223
									± 246
9	—	—	—	—	—	—	—	—	105
									± 164

We have accordingly for the chance (P) that λ does not exceed some value κ

$$P = \frac{\int_0^\kappa e^{-\lambda n}(1 - e^{-\lambda})^m \, d\lambda}{\int_0^w e^{-\lambda n}(1 - e^{-\lambda})^m \, d\lambda} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(14).$$

Expanding the brackets, the numerator and denominator can be integrated as before and an equation obtained for the value of x corresponding to a given value of P. For small numbers of tubes there is no difficulty, though the laboriousness of the work rapidly increases with the number of tubes. Alternatively, writing

$$e^{-\lambda} = x, \quad d\lambda = -\frac{dx}{x},$$

substituting and assuming w to be infinite (14) becomes

$$\frac{\int_{e^{-\kappa}}^1 x^{n-1}(1-x)^m \, dx}{\int_0^1 x^{n-1}(1-x)^m \, dx} = 1 - \frac{\int_0^{e^{-\kappa}} x^{n-1}(1-x)^m \, . \, dx}{\frac{(n-1)!\, m!}{(m+n)!}} \quad \ldots\ldots(15).$$

If now we require the value of κ which corresponds to some assigned probability, we can rewrite (15) as

$$\frac{\int_0^x x^{n-1}(1-x)^m \, . \, dx}{\frac{(n-1)!\, m!}{(m+n)!}} = 1 - P \quad \ldots\ldots\ldots\ldots\ldots\ldots(16).$$

Integrating the numerator of (16) by parts, (taking x^{n-1} as the direct integrand so as to keep the terms all positive) it becomes

$$\frac{x^n}{n}\left\{(1-x)^m + \frac{m}{n+1}\, x\,(1-x)^{m-1} + \frac{m(m-1)}{(n+1)(n+2)}\, x^2 (1-x)^{m-2} \ldots\right\} \quad \ldots\ldots\ldots(17).$$

The series converges fairly rapidly and in rough practice it will be found that a sufficiently good approximation is often reached by putting the bracket equal to unity and solving for x from

$$\frac{x^n}{n} = \frac{(n-1)!\, m!}{(m+n)!}(1-P) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(18).$$

As the sum within the bracket cannot exceed unity, this approximation underestimates x and therefore over-estimates λ, an error on the safe side.

A point of some interest is to consider the problem suggested by (9 *a*) of the last section taking the modal value from (12), *i.e.* putting

$$x = \frac{n}{n+m}$$

(17) then becomes

$$\frac{\left(\dfrac{n}{n+m}\right)^n}{n}\left\{\left(\frac{m}{m+n}\right)^m + \frac{m}{n+1}\left(\frac{n}{n+m}\right)\left(\frac{m}{m+n}\right)^{m-1} + \frac{m(m-1)}{(n+1)(n+2)}\left(\frac{n}{n+m}\right)^2\left(\frac{m}{m+n}\right)^{m-2} + \dots\right\} \quad \text{......(19)},$$

and in the important special case of $m = n$

$$\frac{(\frac{1}{2})^{2n}}{n}\left\{1 + \frac{n}{n+1} + \frac{n(n-1)}{(n+1)(n+2)} + \frac{n(n-1)(n-2)}{(n+1)(n+2)(n+3)} \dots\right\} \quad \text{.........(19 a)}.$$

The series in (19 *a*) is readily summed. Call it $F(n)$. Then

$$\frac{2n!}{n!\,n!} \cdot F(n) = \frac{(2n)!}{n!\,n!} + \frac{(2n)!}{(n+1)!\,(n-1)!} + \frac{(2n)!}{(n+2)!\,(n-2)!} + \dots$$

$$= C_n^{\,2n} + C_{n+1}^{\,2n} + C_{n+2}^{\,2n} \dots$$

$$= \frac{1}{2}\sum_0^{2n} C_r^{\,2n} + \frac{1}{2} C_n^{\,2n}$$

$$= \frac{1}{2} \cdot 2^{2n} + \frac{1}{2}\frac{(2n)!}{n!\,n!}.$$

$$\therefore\ F(n) = \frac{1}{2}\left\{\frac{2^{2n}}{(2n)!} \cdot n! \cdot n! + 1\right\}.$$

$$\therefore\ (19\,a) = \frac{(n-1)!\,n!}{2n!\,2} + \frac{1}{2^{2n+1}\,n} \quad \text{..............................(19 b)}.$$

Substituting in (15) we have

$$P = 1 - \left(\frac{1}{2} + \frac{(2n)!}{n!\,n!} \cdot \frac{1}{2^{2n+1}}\right) \quad \text{..................(19 c)}.$$

Applying Stirling's theorem to the third term it becomes $\dfrac{1}{2}\dfrac{1}{\sqrt{\pi n}}$, which is zero when n is infinite, and then the mode divides the frequency into equal parts.

For usual values of n the approximation is slow. Thus

n	Value of (15)	n	Value of (15)
1	·2500	100	·4718
2	·3125	500	·4874
10	·4119		

Reverting for a moment to the integral in the denominator of (16), we may note that its value can be approximated to by Laplace's method of putting

$$\int y \,.\, dx = Y \int e^{-t^2} \frac{dx}{dt} \,.\, dt.$$

Y being the maximum value of the function within the required range, in this case the value for $x = \frac{n-1}{n+m-1}$, if x is written $\frac{n-1}{n+m-1} + \vartheta$, t^2 is obtained as a function of ϑ and then ϑ as a function of t by reversion of series.

The result to a first approximation only is that the integral transforms to

$$\frac{(n-1)^{n-1}(m-2)^m}{(n+m-1)^{n+m-1}} \int e^{-t^2} \frac{\sqrt{2}}{(n+m-1)\left(\frac{1}{m+1} + \frac{m}{(m+2)^2}\right)^{\frac{1}{2}}} dt.$$

This method of treatment is, of course, well known. We can, however, employ the method of the last section and investigate the properties of $y = x^{n-1}(1-x)^m$, or the distribution of $e^{-\lambda}$, where the range is from 0 to 1.

The mode is at

$$x = \frac{n-1}{n+m-1} = \frac{n-1}{N-1} \quad \text{.....................(19 } d\text{)}$$

and, the moments about the start of the curve being simply successive B functions we easily find

$$\text{mean} = \frac{n}{N+1} \quad \text{...(20)},$$

$$\mu_2 = \frac{n(m+1)}{(N+1)^2(N+2)} \quad \text{..................................(21)},$$

$$\mu_3 = \frac{2n\left[2n^2 - 3n(N+1) + (N+1)^2\right]}{(N+1)^3(N+2)(N+3)} \quad \text{.........(22)}.$$

A comparison of the mean and mode shews again that the curve is skew for all finite values of n and m greater than zero. The transformed curve, $y = y_0 e^{-\lambda n}(1-e^{-\lambda})^m$ is, of course, also very skew, cf. the two distributions shewn in Fig. 3.

Finally we have the case of samples of different sizes.

Thus if N_1 samples each of a_1 c.c. have given n_1 negative and m_1 positive results, N_2 samples each of a_2 c.c. have given n_2 negative and

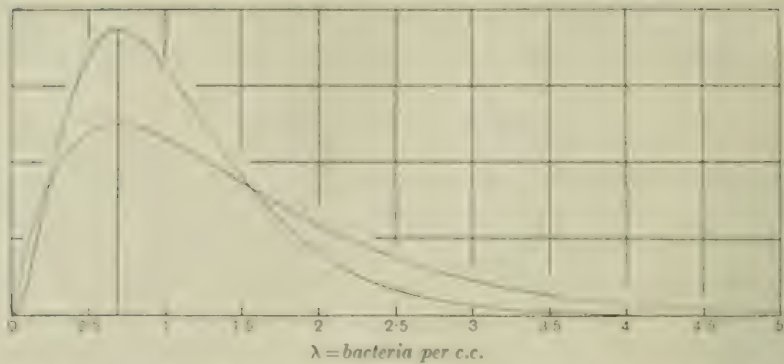

Fig. 3. Chart shewing frequency distributions of bacterial densities for 1 positive out of 2 and 2 positives out of 4 (1 c.c. in each test). The area of each curve is 10 squares.

m_2 positive results and ... N_n samples of a_n c.c. have given n_n and m_n negative and positive results, (14) becomes:

$$P = \frac{\int_0^x \left[e^{-\lambda a_1 n_1}(1-e^{-\lambda a_1})^{m_1} \,.\, e^{-\lambda a_2 n_2}(1-e^{-\lambda a_2})^{m_2} \dots e^{-\lambda a_n n_n}(1-e^{-\lambda a_n})^{m_n}\right] d\lambda}{\int_0^\infty \left[e^{-\lambda a_1 n_1}(1-e^{-\lambda a_1})^{m_1} \,.\, e^{-\lambda a_2 n_2}(1-e^{-\lambda a_2})^{m_2} \dots e^{-\lambda a_n n_n}(1-e^{-\lambda a_n})^{m_n}\right] d\lambda} \quad \dots\dots\dots(23).$$

This expression does not lend itself to simple treatment in the general case but in any particular case it may furnish a solution without much difficulty. The arithmetic may however prove almost unworkable. In an illustration given in Section V only the most probable values of the bacillary density have been approximately determined by an artifice. If the tests are not repeated, only one test being made with a tube of each size, the expression simplifies.

On the general question of the best series of sizes of tubes to use, when the waters to be tested may present so great a range between purity and the reverse that the use of different sizes seems desirable, we may make a few remarks. One obvious condition, strangely overlooked, is that the size of any one sample should be greater than the sum of the sizes of the smaller samples. Otherwise the observer is simply asking for "inconsistencies" in his results. A geometrical series fulfils the required condition, and the Metropolitan Water Board have actually used a geometrical series with the ratio 10 (0·01, 0·1, 1, 10, 100 c.c.). This ratio seems rather a high one. A geometrical series seems also a natural one to use as the chance of an inconsistence is the same

at every point of the series: r being the (ascending) ratio of the series, the chance of an inconsistence between any adjacent pair of samples (the larger giving a negative, the smaller a positive) is $1/(r+1)$.

The various expressions found above enable us to solve all the problems proposed and in the following section we provide a few arithmetical examples of their use.

SECTION V. NUMERICAL ILLUSTRATIONS.

(*a*) *The case of a blank sample.*

If 50 c.c. were tested and found sterile we have from (3)

$$e^{-50\kappa} = 1 - P \text{ or } -50\kappa \log e = \log(1-P).$$

For $P = \cdot 5$ this gives $\kappa = \cdot 01386$.
,, $P = \cdot 99$,, ,, $\kappa = \cdot 09210$.
,, $P = \cdot 999$,, ,, $\kappa = \cdot 13816$.
,, $P = \cdot 9999$,, ,, $\kappa = \cdot 18420$.

So that the chances are even that the source does not contain more than 14 bacilli per litre. It is 99 to 1 that there are not more than 92 per litre, 999 to 1 that there are not more than 138 per litre and 9999 to 1 that there are not more than 184 per litre.

Had 150 c.c., *e.g.* a sample of 100 and a sample of 50 both proved sterile, substitution of 150 for 50 gives for the corresponding values per litre, 5, 31, 46, 62.

(*b*) *One sample is sterile, the other not.*

Suppose 100 c.c. are positive, 50 c.c. sterile. Then using (4) the equation to be solved is

$$1\cdot 5e^{-50\kappa} - \cdot 5e^{-150\kappa} = 1 - P.$$

And we reach:

P	Bacilli per litre	P	Bacilli per litre
·5	21	·999	146
·99	100	·9999	192

(*c*) *Repeated Tests with Tubes of Equal Volumes.*

As a first illustration we take Beveridge and Wanhill's example of 10 samples, each 1 c.c., 7 of which are sterile and 3 show growth.

Here $n = 7$, $m = 3$, $N = 10$ so that by (19 D) and (20) the mode of the x distribution is at ·66667 and the mean at ·63637. That is a source with a density of 405 bacilli per litre (from $e^{-\lambda} = \cdot 66667$) is

the most probable state. Owing, however, to the marked skewness of the distribution, this result is of little service, we must solve (16) for different values of $1 - P$.

If we wish to find the density corresponding to $P = \cdot 5, \cdot 9, \cdot 99$ we have

$$120r^7 - 315r^8 + 280r^9 - 84r^{10} = \begin{cases} \cdot 5 \\ \cdot 1 \\ \cdot 01. \end{cases}$$

Thus, taking the last case, we have

$$f(r) = 120r^7 - 315r^8 + 280r^9 - 84r^{10} - \cdot 01,$$
$$f'(r) = 840r^6 - 2520r^7 + 2520r^8 - 840r^9,$$

and by successive approximation we find that ·29716 is very nearly a root.

Hence from $r = \cdot 29716$ we reach 1213 bacilli per litre: the odds are 99 to 1 that the density of the source does not exceed this value.

The densities corresponding to an even chance and to odds of 9 to 1 are similarly found to be 439 per litre and 802 per litre respectively.

In Table II we give examples of the significance of one or more sterile tubes in a series of 2–4 samples.

TABLE II.

Tables illustrating the significance of one or more blanks in two, three, or four tests on 1 c.c. If the samples are of n c.c., not 1 c.c., divide the numbers of bacilli per litre by n.

Two tests.

The probability is	If the number of blanks is 1	2
0·75 that the bacilli per litre exceed	690	140
0·50 " " " "	1230	350
0·25 " " " "	2050	690
0·01 " " " "	5300	2300
Most probable number per litre	690	nil

Three tests.

The probability is	If the number of blanks is 1	2	3
0·75 that the bacilli per litre exceed	990	400	95
0·50 " " " "	1580	690	230
0·25 " " " "	2390	1120	460
0·01 " " " "	5700	2830	1540
Most probable number per litre	1100	410	nil

Four tests.

The probability is	If the number of blanks is 1	2	3	4
0·75 that the bacilli per litre exceed	1220	610	210	70
0·50 ,, ,, ,, ,,	1840	950	490	170
0·25 ,, ,, ,, ,,	2670	1410	780	350
0·01 ,, ,, ,, ,,	5990	3170	1960	1150
Most probable number per litre	1390	690	290	nil

As stated at the outset, divergences in the numbers of tubes forming different workers' series are too great to allow us to calculate any single table of general service. But the arithmetical examples should suffice to enable any bacteriologist to construct a table covering his own series.

(*d*) *Tubes of different volumes, test not repeated.*

A series of tubes of different volumes is used by several observers. *e.g.* as already mentioned Lelean, and Hewlett. Comment has already been made on the inconvenient character of the series used in each of these cases. That used by the Metropolitan Water Board is a simple geometrical series with a ratio of 10, viz. 100, 10, 1, 0·1, 0·01 c.c. The ratio is high, but it must be admitted that this simplifies the work of calculating the theoretical significance of the results; we have already pointed out that a geometrical series seems the right series to use.

Writing down equation (23) in its simplified form, where $n_r = 0$ and $m_r = 1$ or conversely, expanding, and retaining only the first power of x in the resulting equation we find the following approximate numbers of bacilli per litre:

	100 +, rest −	100 +, 10 +, rest −	100 +, 10 +, 1 +, rest −	100 +, 10 +, 1 +, 0·1 +
$P = 0·5$	72	720	7260	78940
$P - 0·99$	424	4245	42820	470150
Most probable numbers	23	230	2310	24540

The values of λ are nearly, it will be noticed, but not quite, a geometric series.

(*e*) *Repeated tests with tubes of different volumes.*

This involves the determination of the limiting density from (23). As an illustration we take the following series from one of the Reports of the Metropolitan Water Board.

Size of Sample	Source *A* Negative	Source *A* Positive	Source *B* Negative	Source *B* Positive
100 c.c.	308	30	312	21
10 c.c.	333	5	327	6
1 c.c.	336	2	329	4
·1 c.c.	338	0	333	0
·01 c.c.	338	0	333	0

It seemed difficult to obtain in this case even fair approximations to the values of the bacillary density for given values of P. But the following method gives good approximations to the most probable values. Taking the expression

$$y = y_0 e^{-\lambda(a_1n_1 + a_2n_2 + \dots)}(1 - e^{-\lambda a_1})^{m_1}(1 - e^{-\lambda a_2})^{m_2}\dots,$$

$$\frac{1}{y}\frac{dy}{d\lambda} = -(a_1n_1 + a_2n_2 + \dots) + \frac{a_1m_1}{1 - e^{-\lambda a_1}}e^{-\lambda a_1} + \frac{a_2m_2}{1 - e^{-\lambda a_2}}e^{-\lambda a_2} + \dots$$
$$= 0$$

for the most probable value. For Series A we find this gives

$$-34503\cdot18 + 3000\frac{e^{-100\lambda}}{1 - e^{-100\lambda}} + 50\frac{e^{-10\lambda}}{1 - e^{-10\lambda}} + 2\frac{e^{-\lambda}}{1 - e^{-\lambda}},$$

or writing $e^{-\lambda} = x$

$$2\frac{x}{1 - x} + 50\frac{x^{10}}{1 - x^{10}} + 3000\frac{x^{100}}{1 - x^{100}} = 34503.$$

Clearly x is near unity, as the value on the right is so large. Substitute accordingly $z = 1 - x$ for x and expanding this becomes

$$2\frac{1 - z}{z} + 50\frac{1 - 10z + 45z^2}{10z - 45z^2} + 3000\frac{1 - 100z + 4950z^2}{100z - 4950z^2} = 34503.$$

Or, ignoring z^2

$$37{,}555z = 37,$$
$$z = 0\cdot000985,$$
$$x = 0\cdot999015,$$
$$\lambda = 0\cdot965 \text{ per litre.}$$

For Series B we find to a similar approximation $\lambda = 0\cdot838$ per litre, or the water from Source B is most probably slightly purer than that from Source A, though there is no practical difference.

As a glance at the two series suggests, moreover, they are not consistent with each other. Source B, if the better water, should give fewer positives in the small tubes as well as the large. Actually it gives much fewer in the large tube, but more in the small ones. Using the above values of λ, we find for the theoretical as against the actual distributions of positives:

Size of sample	Source A		Source B	
	Actual	Calculated	Actual	Calculated
100	30	31·7	21	27·0
10	5	3·3	6	2·8
1	2	·3	4	·2

It looks as if we were not dealing with a mere chance distribution from water essentially of constant character, but with occasional slight contaminations of the source.

THE BACTERIOLYTIC ACTION OF GLAND EXTRACTS ON TUBERCLE BACILLI.

By A. E. PORTER, *Carnegie Research Fellow.*

(*From the Bacteriological Department, Edinburgh University.*)

The extent to which animal tissues are capable of destroying tubercle bacilli is of great importance, because of the heavy toll of lives which tuberculosis yearly demands, because of the peculiarly resistant nature of the bacillus itself, and the very questionable immunity which it is capable of producing.

The degree of immunity existing in tuberculosis is unknown. The bacillus, like other organisms, contains specific protein, which when inoculated into the animal body is capable of giving rise to protein antibodies, such as precipitins and agglutinins. These were to be expected. Just how far these protein re-actions are an expression of a truer immunity is difficult to say; they may well be regarded as by-products of immunity which have little significance as regards the resistance of the body to infection. Even if the occurrence of tubercle protein in the bloodstream be invariably followed by the advent of antibody, there is no proof that tubercle toxin will call forth antitoxin in the ordinary sense of the term. Tuberculous serum may be toxic, but it has not been found antitoxic. Again, tubercle bacilli may circulate in the blood, but no production of bacteriolysin will follow. Arloing has found that tubercle bacilli, agglutinated by an antiserum, appear to grow even better than if the serum had not been present. The only blood which has been described as bacteriolytic to tubercle is that of *Galeria mellonella.* Sieber and Metalnikoff, who observed this bacteriolysis, attributed it to a somewhat heat resistant ferment, and not to the ordinary mechanism of complement and amboceptor. Treatment with bacterial products may indeed be followed by an increase in the phagocytic power of the blood, but in how far this property may be regarded as advantageous depends entirely on the fate of the bacilli after ingestion. Living bacilli, carried in the bloodstream

by leucocytes, might prove a source of danger rather than an evidence of a means of defence. Leucocytes are, however, known to be peculiarly rich in ferments, and dissolution of many species of bacteria ingested does take place, provided that the particular ferments suited to the bacteria are present.

Power of Tissues against Tubercle.

Tuberculous tissues do appear to exert some destructive influence on the bacilli *in vitro* and *in vivo*. Much has described partially degenerate bacilli in lupus, known as "Much's forms," which have lost their acid-fast properties. Fontes, who also found these forms in tuberculous pus, experimented with extracts of caseous glands on living bacilli. He found that the numbers of the bacilli were thereby reduced, the bacteriolytic substance remaining active for 120 days. Wolff has found Much's forms in the glands of otherwise non-tuberculous children, *post mortem*. Of all human tissues, the skin appears to exert the most remarkable influence on tubercle bacilli growing in it. Not only can it rob the bacilli of their characteristic staining properties, but, as the careful work of Griffiths has so clearly shewn, the virulence of the bacillus is profoundly altered. Lupoid material injected into guinea-pigs has sometimes given rise to no lesions whatever, although the living, but non-pathogenic bacilli could be afterwards recovered and cultivated from glands and omentum. Guinea-pigs treated by Spiro with extracts of tuberculous lymphatic glands were rendered thereby more resistant to a later experimental tuberculosis. Trudeau, in similar experiments, obtained partly favourable results with human tuberculous gland preparations. There is also the work of Kitasato in 1892, proving that the majority of bacilli in sputum, though staining well, are already dead.

That normal tissues can exert an anti-tuberculous influence has also been demonstrated. Markl has recorded a complete bacteriolysis of tubercle bacilli both within and outside phagocytes in peritoneal exudates in guinea-pigs which he examined at intervals within 24 hours after intraperitoneal injection of the bacilli. Kling has seen some bacteriolysis of tubercle bacilli when these were treated with leucocytic extracts *in vitro*. Calmette and Guerin lowered the virulence of the bacilli by means of bovine bile, as did Bartel, Neumann, and Leimsner by using proteolytic ferments, lipoids, and fatty soaps extracted from the spleen, liver and lymphatic glands. Neumann and Wittgenstein

found that in normal lymphatic glands, pancreas, liver, or ovary, the virulence of tubercle bacilli was either entirely lost, or at least so much lowered that the bacilli after intravenous injection were no longer able to cause generalised but only local infections. Defibrinated blood, or lung tissue, had no such influence. Wittgenstein also found that the life of tuberculous animals could be prolonged by treatment with ovarian substance. Schroeder found that experimental tuberculosis ran a more chronic course in guinea-pigs treated with spleen substance. Withe and Zeublin investigated the influence of some fresh and autolysed gland extracts on the virulence of tubercle bacilli, and found that autolysed extracts of rabbit's lung and liver exerted a marked influence. Unfortunately they do not record the exact acidity of the autolysed extracts, although they mention that the liver extracts were very acid. These authors did not regard any bacteriolysis as having taken place, as the bacilli were unchanged in form, and staining properties. Deycke and Much have described a complete dissolution of tubercle bacilli in certain brain substances, such as cholin, neurin, and lecithin, which has been attributed by Tessen and Rabinowitsch to alkalinity alone. Sieber and Metalnikoff have found some strains of tubercle bacilli actually dissolved by lipase. Perhaps the best, because the best known, demonstration of anti-tuberculous power on the part of the tissues is afforded by the selective tendency so characteristic of tuberculous infection. One species is preferred to another species; one organ to another organ. Take, for example, the comparative immunity enjoyed by the rabbit's liver in comparison with its highly susceptible lungs, or the remarkable rarity of tuberculosis in the sheep in comparison with its frequency in the ox.

Nature of the Bacillus.

The highly resistant qualities of the tubercle bacillus, its slow growth, and its acid-fast properties are believed to be due to its envelope, which contains a high percentage of fats and waxes, and which is only penetrable with difficulty by bacteriolytic substances, food material and solvents. If this fatty envelope could be first dealt with, the bacillus itself would present less difficulty. This fatty substance has been shewn to consist in great measure of fatty acid esters of glycerine and especially of higher alcohols. Baudran found the percentage of fatty material varying in different strains from 36 % to 44 %. Kresling extracted 38·95 % of total "fat" from the bacillus, of which 39·1 %

was present in the form of fatty acid esters of higher alcohols alone. With the lipases which the animal body contains and by means of which it splits esters, it might be possible to break down the envelope and reach the bacillus. A bacteriolysis of the tubercle bacillus might be effected through esterases in this way, either by physical disruption, by increasing solubility, or by the production of acid.

Lipolysis and Bacteriolysis.

The two methods of destroying bacilli which most readily suggest themselves as within the scope of the defences of the body, where heat is in the case of the pathogenic organisms hardly applicable, are certainly on the one hand the production of acid or alkali, on the other hand solution of some constituent of the bacillary wall. Take, for example, the destruction of the colon bacillus in bladder infections when the urine becomes alkaline, or the effect of lactic acid in sour milk on the organisms of the intestine, or again the action of the acid stomach contents upon cholera spirilla. Many antiseptics are solvents, especially fat solvents; for example, alcohol, ether, carbolic acid. In ferment action, more particularly proteolytic and amylolytic action, the body has the power of promoting solubility. This is not so much the case in lipolysis, where the end products, fatty acid and alcohol, are often both highly insoluble. These higher alcohols, notably cholesterin, are, however, either soluble or at least emulsifyable in the fatty acids themselves. The action of free fatty acids upon these alcohols is very remarkable. Although no ester has formed as can be proved by titration, a curious physical change takes place. Add solid fatty acid to warm water in which insoluble cholesterin crystals are lying, the water becomes milky and the cholesterin crystals disappear. Even solid fatty acid falling down in cold water with a clear supernatant fluid, will, if in small pieces and shaken, be taken up into a milky emulsion on the addition of tubercle bacilli, which contain free alcohol. It is interesting that Cramer, Feiss, and Bullock find that "mixtures" of fatty acid and cholesterin stain differently to their esters also differently to either alone. Whether such mixtures represent a solution of the alcohol in the acid, or a loose chemical bond, the ester gives firm union, insolubility and impenetrability; the mixture, from its emulsion-forming tendencies, rather gives penetrability and fine division.

The body has much power of actually dissolving bacteria other than tubercle, both leucocytes and the body fluids have been proved

to possess this power. The actual dissolution of acid-fast bacilli is not easy to observe in body fluids, but it has been abundantly proved that though actual dissolution is so difficult and slow, bacilli have been killed by the tissues even while remaining perfect in form and staining properties. Acid-fast bacilli killed by acid *in vitro* are apparently unchanged in form and staining properties. Lipolysis is accompanied by the production of acid, which may be a low, soluble fatty acid, or a high, insoluble one. Lipolysis requires not only ferment and fat but also an activating agent. Activation is believed to depend upon a removal of the inhibitory products of ferment action. Thus $CaCl_2$, recommended by Kanitz, has been shewn by Pekelharing to act by forming insoluble soaps of calcium, and so by precipitation to remove the products which are hindering further action. The action of bile salts is quite opposite to this; by promoting solubility, especially in the duodenum, they get rid of the end products of lipolysis by their absorption through membrane. This suggests that should fatty acids be absorbed by tubercle, no other activator would be required in the lipolysis of the fats surrounding the bacillus. The milky emulsion just described which takes place when tubercle bacilli are shaken in water with insoluble fatty acids demonstrates that tubercle bacilli can take up these acids. In another communication (Porter, *This Journal*, XVI. p. 66) on the sensitiveness of tubercle bacilli to acid, I have shewn that bacilli are actually killed by these higher fatty acids which though insoluble in the watery medium round the bacillus are yet able to penetrate its fatty envelope when applied to it. If this be so, how much more easily could the bacillus be killed by fatty acids formed in the envelope itself during lipolysis, even though its form and staining reactions remain unchanged. Moreover a preliminary lipolysis may give access to proteolytic ferments which produce also soluble fatty acids, and have greater solvent properties.

With these considerations in view, I was led to study more fully the distribution of lipases, and to examine a number of organs for wax-splitting power, on account of the presumably great importance of these ferments to the resistance of the body against tuberculosis. The results of this investigation have been published elsewhere (*Biochemical Journal*, 1916, vol. X. p. 523) but are briefly indicated in the following tables for purposes of comparison.

As lipolysis is accompanied by acid production it was necessary to estimate the sensitiveness to acid, inorganic and fatty, of the bacilli under investigation. It was found (*This Journal*, XVI. p. 66) that tubercle

bacilli were killed in the presence of $n/10$ acid, but could resist weaker concentrations of acid for 24 hours, while the other acid-fast bacilli could not resist $n/80$.

Extracts of the organs tested for esterases, have also been examined for bactericidal power against tubercle bacilli.

Methods.

Extracts were made from the following organs:

	Human	Ox	Sheep	Pig	Cat	Rabbit	Guinea pig
Pancreas	×		×	×			
Liver	×	×	×	×	×	×	×
Lung	×	×	×	×	×	×	×
Spleen	×	×	×	×	×	×	×
Kidney	×	×	×		×	×	×
Brain	×	×	×	×			
Suprarenals	×	×	×	×			
Thyroid	×	—	×	×			
Thymus	×	×	×	×			
Lymphatic glands ...	×	×	×	×			
Haemolymph glands	—	—	—	—			
Pituitaries ...	—	×	×	×			
Bone marrow ...	—	×	—	×			
Salivary glands ...	×	×	—	×			
Skin	×	—	—	—			

The method of extraction adopted was that recommended by Kanitz, and is very simple. Glands were minced down, and pure glycerin added in the proportion of one-third gland and two-thirds glycerin. After two days' contact, the extract was strained through gauze, as lipases are easily injured by filtration.

The best medium known for extracting lipase from tissues is glycerin. Lipase is soluble in pure glycerin, as also are salts. Fatty acids, alcohols and esters are only very slightly soluble, but enough to aid lipolysis and also titration of these usually difficultly soluble substances. Glycerin also preserves the lipase by abstracting water, which injures the ferment. Glycerin is indeed well known as preservative, and anti-putrefactive, being used in commerce, for example in rennet preparations, etc., for that purpose. Hawthorn thought glycerin bactericidal to tubercle, but Fontes could not confirm this. Bacilli, seven days in 50 % glycerin at 38·5° C., did not lose in vitality or pathogenicity, also sputum kept by him for a year in glycerin, did not putrefy, but preserved the form and staining properties of the bacilli perfectly. It is doubtful how far the bactericidal influence which has

been ascribed to glycerin depends upon the glycerin itself. Glycerin is very liable to decomposition during purification by distillation, and may contain acrylaldehyde, and acrylic acid, which are bactericidal. This can be guarded against by testing for acidity. Again, if glycerin is boiled some decomposition may take place with the formation of these bactericidal products. At the beginning of this research all glycerin was boiled before use, with the intention of destroying any glycerin tolerant organisms which might be present, with the result that often embarrassingly bactericidal properties were developed. It was later heated not above 100° C. with satisfactory results. Glycerin is hygroscopic, absorbing water up to half its bulk, and for this reason should not be too concentrated when used with organisms. In the following experiments the glycerin present in the bactericidal mixtures was always less than 25 %, it having been previously ascertained that 50 % of glycerin was not bactericidal. Although ferment solutions extracted from organs are usually difficult to preserve from putrefaction, the glycerin extracts made from organs in this investigation all became sterile within a few days. That this did not depend on the direct action of the glycerin on the organisms, but rather on its preservative influence on the bacteriolytic substances present, was proved by the varying rate at which the organs became sterile. Liver became sterile at once; lung was perhaps the slowest of all. This sterility allowed of certainty in ascribing lipolytic action to the organ from which the extract was made.

Seven strains of human tubercle bacilli were used, *i.e.* H 1, 20, 28, 67, 70, 79 and Arloing-Courmont strain, also four strains of bovine tubercle bacilli, B 1, 4, 43, and 164, and five other acid-fast bacilli (Mist, Timothy Grass, Korn, Rabinowitsch, and Smegma). An emulsion was made in half-strength physiological saline solution. To do this, it was necessary in the case of the human strains, except Arloing-Courmont, to shake the bacilli in a shaking machine for two or three hours; the bacilli if shaken overnight may all be killed, so it is advisable not to overshake. The bovine strains were also occasionally shaken, though this was less necessary, as bovine tubercle bacilli usually emulsify better.

Bacteriolysis was tested in the following way: in each of a series of small sterile tubes were placed 0·25 c.c. of a different gland extract (glycerin $\frac{2}{3}$), then 0·5 c.c. half-strength physiological saline solution, then 0·25 c.c. bacillary emulsion. A part of the saline solution was sometimes replaced by sufficient $n/10$ NaOH to neutralise the known

acidity of the gland extract, or by weak $CaCl_2$ solution, or by bile. The gland extracts were always slightly acid, but not sufficiently so to affect the bacilli (Porter, *This Journal*, XVI. p. 66). The acidity of the bacteriolytic mixtures increased during the $2\frac{1}{2}$ and 24 hours of contact, at 37° C., or room temperature, but usually the resulting acidity was scarcely sufficient to account for the degree of bacteriolysis; however a part of the acid produced may have been absorbed by the bacilli, that is to say retained by the bacilli and not titrated. The addition of lecithin increased the bacteriolysis, but the acid split from it was sufficient to account for this. Esters scraped from egg medium together with the bacilli (and especially human strains are somewhat apt to eat into the medium and soften it) will, through splitting, increase the acid present. Bacteriolysis was apparently unaffected by the presence of weak $CaCl_2$, or of bile. When the bacteriolytic mixtures had been in contact, usually for 24 hours at 37° C., four drops were taken from each with a sterile pipette and inoculated on some medium. The Arloing-Courmont strain, and the acid-fast bacilli, other than tubercle, were inoculated on 3 % glycerin agar. As the other strains would not grow well on glycerin agar, Dorset was first used. This had to be discarded, as egg medium contains very many esters, lecithin, other glycerides, and cholesterin esters, so that strong lipase mixtures, notably those containing pancreas extract, ate into and liquefied the medium. B43 refused to grow on anything but egg medium, but those of the other strains which refused glycerin agar, yet grew well on glycerin-cholesterin agar. The bacteriolytic influence of gland extracts, like the lipolytic, slowly deteriorated, so that it was much lower in a few weeks' time, and practically non-existent in 2–3 months. Thus old inactive extracts, containing the same acid and glycerin, were available to control their former activities when fresh. It must be admitted that tubercle bacilli, though far less sensitive to acid than other acid-fast bacilli, are yet more sensitive to lipolytic gland extracts, either because they contain more fat (tubercle 10–14 %, Mist 16 %), or because the fats are of a different nature. Dead bacilli from lipolytic mixtures, for the most part stained perfectly with Ziehl-Nielssen and Gram stains, though a certain percentage lost their acid fast properties. Proteolytic ferments affect the staining properties of acid fast bacilli much more profoundly than do lipolytic. Dead bacilli, staining perfectly, rather suggest the effect of acid alone.

The following table shewing the bacteriolytic activity of the gland extracts investigated, also contains, for comparison, an indication of

Gland extract	Esterases present in gland extract: Glycerin esterases	Lecithase	Higher esterases	Bacteriolytic power of gland extract on tubercle bacilli
Human Pancreas	+	+	weak	complete
Ox ,,	+	+	+	,,
Sheep ,,	+	+	+	,,
Pig ,,	+	+	+	,,
Human Liver	+	+	weak	,,
Ox ,,	+	+	+	,,
Sheep ,,	+	+	weak	partial
Pig ,,	+	+	+	complete
Cat ,,	+	+	+	,,
Rabbit ,,	+	+	+	,,
Guinea-pig ,,	+	+	weak	partial
Ox Thymus	+	+	+	complete
Sheep ,,	+	+	+	,,
Pig ,,	+	+	+	,,
Human Lymph Glands	weak	weak	weak	,,
Ox ,, ,,	+	+	+	,,
Sheep ,, ,,	+	+	+	,,
Pig ,, ,,	weak	weak	weak	,,
Human Suprarenals	0	,,	,,	partial
Ox ,,	+	,,	,,	complete
Sheep ,,	weak	,,	,,	partial
Pig ,,	,,	,,	,,	,,
Human Spleen	0	+	0	0
Ox ,,	weak	+	0	0
Sheep ,,	0	+	0	0
Pig ,,	+	+	0	partial
Cat ,,	weak	+	0	,,
Rabbit ,,	,,	+	0	0
Guinea-pig ,,	,,	+	0	0
Human Kidney (fatty)	+	weak	weak	partial
Ox ,, ,,	0	,,	0	0
Sheep ,, ,,	0	,,	0	0
Cat ,, ,,	+	,,	weak	partial
Rabbit ,, ,,	weak	,,	0	0
Guinea-pig ,, ,,	0	,,	0	0
Human Brain	weak	+	0	partial
Ox ,,	0	+	0	,,
Sheep ,,	0	+	0	0
Pig ,,	0	+	0	0
Human Thyroid	0	weak	0	0
Ox ,,	+	+	+	complete
Sheep ,,	+	+	weak	0
Pig ,,	0	weak	0	0
Human Lung	0	trace	0	0
Ox ,,	0	weak	0	0
Sheep ,,	weak	,,	0	0
Pig ,,	,,	,,	0	0
Cat ,,	+	,,	0	partial
Rabbit ,,	0	trace	0	0
Guinea-pig ,,	0	,,	0	0
Human Skin	+	weak	+	complete
Ox Bile	+	0	0	0
Pig Salivary Glands	0	weak	0	0
Ox Bone Marrow	+	,,	weak	partial
Ox Pituitaries	weak	,,	,,	,,
Sheep ,,	0	,,	0	0

the esterases present in the same extracts. This table gives a brief summary of another paper wherein the distribution of esterases in the different organs is discussed in fuller detail (*vide* Porter, *Biochemical Journal*, 1916, x. p. 523).

SUMMARY.

The results obtained point to a consistent relationship between lipolytic activity and bacteriolytic power on tubercle bacilli.

The least bactericidal extract was lung extract; the most powerful was pancreas extract.

Liver, thymus and lymphatic glands were strongly bactericidal.

Other organs, suprarenal glands, pig and cat spleen, human and cat kidney, human and ox brain, ox thyroid, cat lung, ox bone marrow and ox pituitary glands were found to be bactericidal to a lesser degree.

The human skin extract examined for bactericidal properties was fatty and cloudy in appearance and exceptionally rich in esterases[1]. Even if exceptional in its esterase activity this sample of skin bears out the relationship between lipolysis and bacteriolysis of tubercle bacilli in a striking way, as it was also extremely bactericidal.

No difference was noticed between bovine and human tubercle bacilli in susceptibility to any gland extract examined.

Other acid-fast bacilli, though on the whole less susceptible than tubercle bacilli to the influence of these extracts, were bacteriolysed by them. They were also killed by one lung extract (pig's) which contained an unusually large amount of olein lipase and which had no effect on tubercle bacilli.

I wish to express my thanks to Dr W. Cramer for his kind advice and encouragement, to Dr Cobbett for his kindly criticism, to Professor Shennan and Dr Miller for human material, and to Dr Wang for several strains of tubercle bacilli.

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[1] Two other skin extracts were not cloudy and were comparatively poor in esterases, possibly due to the condition of the sweat glands at death.

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THE SENSITIVENESS OF TUBERCLE AND OTHER ACID-FAST BACILLI TO ACIDS.

By A. E. PORTER, *Carnegie Research Fellow.*

(*From the Bacteriological Department, Edinburgh University.*)

It was desired to determine the minimum amount of acid which would kill tubercle and other acid-fast bacilli, incidentally to note any distinction between human and bovine tubercle bacilli and between tubercle and other acid-fast bacilli in respect to sensitiveness to acid, and to find whether inorganic acid were more powerful in action than organic acid, and whether insoluble fatty acids were also effective.

Seven strains of human tubercle bacilli were used (H 1, 20, 28, 67, 70, 79 and Arloing-Courmont strain), also four strains of bovine (B 1, 4, 43 and 161) and five other acid-fast bacilli (Mist, Timothy Grass, Korn, Rabinowitsch and Smegma).

Method of Testing Sensitiveness to Acid.

In order to test the sensitiveness of these eleven tubercle strains, and the five other acid fast bacilli to acid, standard solutions, *n* 2, *n* 5, *n* 10, were made up of sulphuric, acetic and citric acids. Corresponding solutions of stearic, palmitic and oleic acids in benzene were also made, of which, after being very slightly warmed to completely dissolve, 0·25 c.c. were measured out into small tubes, the benzene being evaporated off through the cotton wadding on a stove. Half strength physiological saline solution 0·75 c.c. was then added (sometimes 0·25 c.c. of this saline was replaced by glycerin which, dissolving a trace of fat, can thereby act as carrier). The solid stearic acid and palmitic acid were then broken up into as small pieces as possible. This was done by drawing the mass over the glass surface of the tube above the saline, heating the glass and breaking the acid up with a strong needle. Similarly, 0·25 c.c. of the soluble acids, sulphuric, acetic and citric,

was placed in tubes, 0·5 c.c. of saline solution added, and to all tubes 0·25 c.c. of bacillary emulsion. For bacilli other than tubercle 0·125 c.c. and 0·25 c.c. of $n/10$ acid only, was used. After 24 hours' contact at 37° C., and at room temperature, 4 drops of tubercle or 3 drops from the other bacillary-acid mixtures were taken and inoculated on egg-medium in the case of tubercle, or 3 % glycerin agar in the case of other bacilli. The results were strikingly uniform and showed a marked difference between tubercle and other acid-fast bacilli.

Table Illustrating Sensitiveness of Tubercle Bacilli.

		Acidity	Growth
1.	0·25 c.c. $n/2$ H_2SO_4 + 0·5 c.c. 0·4 % NaCl + 0·25 c.c. tubercle emulsion	$n/8$	0
2.	0·5 c.c. $n/5$,, + 0·25 c.c. ,, + ,, ,,	$n/10$	0
3.	0·75 c.c. $n/10$,, + ,, ,, + ,, ,,	$n/13$	+ +
4.	0·25 c.c. $n/5$,, + 0·5 c.c. ,, + ,, ,,	$n/20$	+ +
5.	0·25 c.c. $n/10$,, + ,, ,, + ,, ,,	$n/40$	+ +
6.	0·25 c.c. $n/2$ acetic + ,, ,, + ,, ,,	$n/8$	0
7.	0·5 c.c. $n/5$,, + 0·25 c.c. ,, + ,, ,,	$n/10$	0
8.	0·75 c.c. $n/10$,, + ,, ,, + ,, ,,	$n/13$	+ +
9.	0·25 c.c. $n/5$,, + 0·5 c.c. ,, + ,, ,,	$n/20$	+ +
10.	0·25 c.c. $n/10$,, + ,, ,, + ,, ,,	$n/40$	+ +
11.	0·25 c.c. $n/2$ citric + 0·5 c.c. ,, + ,, ,,	$n/8$	0
12.	0·5 c.c. $n/5$,, + 0·25 c.c. ,, + ,, ,,	$n/10$	0
13.	0·75 c.c. $n/10$,, + ,, ,, + ,, ,,	$n/13$	+ +
14.	0·25 c.c. $n/5$,, + 0·5 c.c. ,, + ,, ,,	$n/20$	+ +
15.	0·25 c.c. $n/10$,, + ,, ,, + ,, ,,	$n/40$	+ +

The 11 strains of tubercle bacilli tested, were all killed in 24 hours in the presence of $n/10$ sulphuric, acetic and citric acids, but they were not killed in 24 hours in the presence of $n/13$.

These bacilli could also be killed by stearic, palmitic and oleic acids, if the solid acids were finely divided and sufficiently mixed with the bacillary emulsion. A curious phenomenon occurred when bacilli and these last three acids were mixed; a thick milky emulsion formed, in which the acid was prevented from separating out from the saline solution as it would otherwise have done. Bacteriolysis with these acids, though sometimes complete, was more often partial, depending as it did on the insoluble acid reaching the bacillus. No difference could be observed between bovine and human tubercle bacilli in their sensitiveness to acid.

Other Acid-fast Bacilli.

Method as before, 24 hours' contact.

									Acidity	Growth
1.	0·25 c.c.	$n/10$ H_2SO_4	+0·5 c.c.	0·4 % NaCl	+	0·25 c.c.	bacil. emulsion		$n/40$	0
2.	0·125 c.c.	,, ,,	+0·625 c.c.	,,	+	,,	,,		$n/80$	0
3.	0·25 c.c.	,, acetic	+0·5 c.c.	,,	+	,,	,,		$n/40$	0
4.	0·125 c.c.	,, ,,	+0·625 c.c.	,,	+	,,	,,		$n/80$	0 or trace
5.	0·25 c.c.	,, citric	+0·5 c.c.	,,	+	,,	,,		$n/40$	0
6.	0·125 c.c.	,, ,,	+0·625 c.c.	,,	+	,,	,,		$n/80$	0 or trace

Compared to tubercle bacilli, other acid-fast bacilli are very much more sensitive to acid; they are killed in 24 hours in the presence of $n/80$ acid. On the other hand they seem less affected by insoluble fatty acids; carefully mixed with stearic, palmitic and oleic acids, the majority commonly survive.

Conclusions.

In sensitiveness to acid, both organic and inorganic acid, a very great difference between tubercle and other acid-fast bacilli was demonstrated. Tubercle bacilli were killed in 24 hours by $n/10$ acid, but could resist more dilute acid; other acid-fast bacilli were killed in $n/80$ acid. The Arloing-Courmont strain, although so different from a typically human bacillus, in cultural characteristics (rapid growth, not eating into media, soft, damp appearance), in emulsifying properties, and in its poor acid fastness, large size (and non-pathogenicity), yet shewed its tubercular nature in this faculty of resistance to acid. Although apt to be partly decolourised by acid, presenting a beaded appearance, it was not killed by a weaker concentration of acid than $n/10$. No difference could be noticed between the action of inorganic and soluble organic acid upon these bacilli. Between soluble and insoluble fatty acid there was naturally some difference, the effect of the latter depending on its reaching the bacillus. If in a fine emulsion and shaken with the bacilli bacteriolysis did occur. Acid-fast bacilli, other than tubercle, although more sensitive to lower acids, were less affected by insoluble fatty acid. No difference in sensitiveness could be detected between human and bovine bacilli.

I wish to express my thanks to Dr Bayon for the strain of Arloing-Courmont tubercle bacilli, and to Dr Wang for eight of the other tubercle strains used.

ON CHILD MORTALITY AT THE AGES 0–5 YEARS, IN ENGLAND AND WALES[1].

By Sir ARTHUR NEWSHOLME, K.C.B., M.D.,

Medical Officer to the Local Government Board.

I. CIRCUMSTANCES OF ENVIRONMENT FAVOURING EXCESSIVE CHILD MORTALITY.

Maternal Ignorance.—Lack of Medical Care and Nursing.

In the preceding reports on infant mortality in counties during 1908 (Cd. 5263), in 241 urban areas in 1907–10 (Cd. 6909), and in Lancashire (Cd. 7511), the chief environmental circumstances favouring excessive infant mortality have been enumerated and to some extent discussed.

These circumstances are complex and numerous, and it is not unreasonable that differences in opinion should be held as to their relative importance. It is clear also that one set of adverse circumstances may lead to excessive infant mortality in one area, and another set in a second area.

These general remarks apply with equal force to the total child mortality at ages 0–5.

Maternal ignorance is sometimes regarded as a chief factor in the causation of excessive child mortality. It is a comfortable doctrine for the well-to-do person to adopt; and it goes far to relieve his conscience in the contemplation of excessive suffering and mortality among the poor.

This doctrine has found favour in occasional official reports and in miscellaneous addresses. It embodies an aspect of truth, but it is mischievous when it implies, as it sometimes does, that what is chiefly required is the distribution of leaflets of advice, or the giving of theoretical instruction as to matters of personal hygiene.

[1] Extracted from the *Forty-fifth Ann. Rep. of the Local Govt. Board*, 1915–16. Supplt. containing a Report on Child Mortality at ages 0–5, in England and Wales (Cd. 8496). H.M. Stationery Office. London, 1916. Price 1s. net.

There is little reason to believe that the average ignorance in matters of health of the working-class mother is much greater than that of mothers in other classes of society. Furthermore, it would appear that working class mothers give their infants the supremely important initial start of breast feeding in a larger proportion of cases than do the mothers in other stations of life.

The mothers in both classes may be ignorant: in both there is deficient training in habits of observation, especially in regard to the beginnings of illness; but the mother in comfortable circumstances is able to ensure for her infant certain advantages which the infant of the poorer mother often cannot obtain. What are these?

(1) The well-to-do mother is commonly able to devote herself to her infant and have assistance in this duty; the working-class mother is single-handed, and has also to perform, unaided, all the duties of her household, including the washing and cooking for her husband and herself and possibly for several children.

(2) The well to-do mother is commonly able to ensure that the milk for her infant is purchased under the best circumstances, is stored in a satisfactory pantry, and is prepared under cleanly conditions. The working-class mother often is supplied with stale impoverished milk, may have no pantry, and, except when suckling her infant, is handicapped at every stage in the cleanly preparation of her infant's food.

(3) If the well to-do mother is ill, adequate medical and nursing assistance is at once available, and the child's welfare can be safeguarded; if the working-class mother is ill, the child usually must suffer with its mother.

(4) If the child of the well-to-do mother falls ill, everything that good nursing and medical attendance can furnish is commonly at once available; for the child of the working class mother the state of matters is remote from this ideal. Facilities for medical attendance and nursing vary greatly in different districts. In London, in small towns, and in rural districts, the nursing assistance provided by district and county nursing associations is usually more generally available than in industrial towns. Prompt medical assistance is of great importance. It is often not available for children of wage earners, and particularly for the children of unskilled workers.

(5) Infants and nursing mothers are very rapidly influenced by their environment. This environment is complex. The mother is the main element in the environment of the infant. If she is overworked

and suffering from chronic fatigue her infant must suffer; directly, because the mother's milk under these circumstances is liable to be impoverished or otherwise unwholesome; or indirectly, owing to insufficient attention to the infant. The infant of the well-to-do mother is less likely to suffer in either of these ways.

(6) Not only are the milk supply, and the storage and preparation of artificial food, important parts of the environment of the infant, but also the housing conditions of the family, and the sanitary conditions of the back yard and of the street in which the house is situate. The superiority of the circumstances of the one mother and infant over those of the other in these respects is obvious.

There is no reason to assume that the one mother is more ignorant than the other. But the ignorance of the working-class mother is dangerous, because it is associated with relative social helplessness. To remedy this what is needed is that the environment of the infant of the poor should be levelled up towards that of the infant of the well-to-do, and that medical advice and nursing assistance should be made available for the poor as promptly as it is for persons of higher social status.

The assistance given will include advice, but it will be the advice which a medical practitioner gives to his patient; which a health visitor gives as to personal hygiene; and which a sanitary inspector gives to a householder. It should include also the advice given by a trained midwife, who is in a favourable position to secure the adoption of her advice by the mother. Such advice is becoming available to a steadily increasing extent, but in some industrial towns a majority of the midwives are still untrained women, who are not competent to give the best advice.

Fecklessness of Mothers.

Probably more important than actual ignorance is carelessness or fecklessness of mothers. In the essential duty of breast feeding the infants of the poor are better served than those of the well-to-do; but, for the reasons set out in the preceding paragraphs, carelessness in other respects in the poor mother is fraught with much greater risk to the infant than corresponding carelessness among the well-to-do.

This carelessness is being diminished by the influence of public opinion and of the example of other mothers. This is one of the not least important ways in which a Child Welfare Centre exercises an important influence for good. Not only is the mother influenced by

the medical advice given, but she is subjected to the stimulus of comparison of her child with the children of other mothers, and to the valuable influence resulting from the evidence of active interest in her child. The systematic visits to the home of a tactful and judicious health visitor confirm this effect.

Intemperance.

That intemperance either in husband or wife is a serious cause of excessive infant mortality is certain. On p. 80 of my *Second Report on Infant Mortality* was given a diagram showing the almost complete correspondence in a long series of years between the annual curve of infant mortality, of proceedings for drunkenness in terms of population, and of *per capita* consumption of beers and spirits; and this general coincidence for the whole country fits in with the experience of individual families.

The close relationship between intemperance and excessive child mortality is not difficult to detect in the experience of different towns. There can be no hesitation in ascribing to this cause an important share in the causation of the excessive child mortality in such towns as Burnley, Wigan, Middlesbrough, Barnsley, Stoke-on-Trent, Liverpool, and Preston.

If abstinence from alcoholic drinks could be enforced in these and many other towns in which child mortality is excessive, their experience in this respect undoubtedly would rapidly improve.

But intemperance is a symptom of social evil as well as its cause. It not only results from example and habit acting on an individual of feeble will power, but it is also a common result of the toxaemia of over fatigue, the habit of excessive drinking being acquired in the foolish attempt to counteract fatigue by this means. Excessive drinking is a product of uninteresting surroundings, and more particularly of bad housing and of domestic discomfort. The consideration of intemperance, therefore, cannot be separated from that of housing conditions, and in the search for the easiest point at which to break the vicious circle of influences dragging parents and children down, there is need in some instances for direct attack on intemperance, and in others for equally vigorous attention to the avoidance of chronic over-fatigue, to improvements in housing and to the provision of wholesome means of recreation.

Poverty.

Poverty in towns undoubtedly favours excessive child mortality. Child mortality is high among the poor and low among the well-to-do. It is highest in the poorest wards in any given town and in the poorest parts of a given ward.

Some of the reasons for this association of poverty with excessive child mortality have been enumerated on p. 70. They include unsatisfactory milk supply, bad housing, deficient medical care and nursing. In particular, the fact that poverty implies living in the most densely populated and the least sanitary parts of a district has important bearing on the excessive child mortality associated with poverty. Some of these factors are discussed in fuller detail in Chapter XIII of the *Second Report on Infant Mortality.*

Poverty is a complex phenomenon, varying in composition in different experiences. To speak of its abolition by the direct application of money as the most efficient means for reducing child mortality is as unscientific as to study the properties of oxygen exclusively in a chemical compound containing oxygen along with other elements. Poverty in one instance may be due to insufficient earnings of the parent, and then additional money or its equivalent is required. Poverty may also be caused by intemperance or gambling or improvidence. Here the giving of money may intensify the evil; though even here assistance for the victims of parental misconduct cannot be withheld although the reform of the parent is not secured

The relative child mortality in the towns reported on gives numerous illustrations of the fallacy of looking at poverty as a simple element. In the mining areas there is nearly always excessive child mortality, although wages are good. Here it is necessary to consider bad housing, a low standard of cleanliness, and the existence of secondary causes of poverty, as responsible in many instances for the destruction of child life. Among these secondary causes of poverty, gambling, intemperance, and improvidence occupy an important place. The moral and the physical causes of poverty act and interact.

In many other areas it is difficult to disentangle cause and effect when sickness and poverty concur. Parental sickness is an extremely important cause of poverty, and excessive child mortality occurs under such conditions. For this sickness bad housing or insanitary conditions of work may be responsible; and so the chain of causation lengthens.

Such a chain presents the hopeful characteristic that its fracture at any point may have effects along each link of the chain.

Overcrowding on Area

is an important determining influence of excessive child mortality. Infectious diseases are more common in densely populated areas; and when this dense aggregation, as frequently happens, is associated with such sanitary defects as bad scavenging, the persistence of privies and pail closets and of unpaved yards and streets, there is always excessive diarrhoea and pneumonia, if not also enteric fever.

Size of Town.

As a rule child mortality is heavier in the larger than in the smaller towns.

That there is, however, no necessary connection between the size of a town and the amount of loss of child life is evident from the numerous examples scattered through the preceding pages. It is only necessary to recall a few illustrations. Newcastle-on-Tyne (199) has a lower child death-rate per 1000 births than Gateshead (211) or Blaydon (228); Darlington (153) a lower child death-rate than Stanley (223); Halifax (151) and Bradford (174) a lower death-rate than Barnsley (241); Birmingham (200) a lower death-rate than Bilston (237), Tipton (222), or Walsall (216); Derby (143) a lower death-rate than Ilkeston (207); Cardiff (170) a lower death-rate than Aberdare (198); and Newport (168) than Blaenavon (217).

Overcrowding in Rooms.

The true test of density of population is the population per room in each tenement. In the following tables the forty great towns and the forty smaller towns having the highest and the lowest child mortality are given, and their relative condition as to overcrowding of rooms (in the non statutory sense of more than two persons per occupied room) is shown. It will be seen that in the large and in the small towns in which the child mortality was low, the proportion of overcrowded tenements was low. Hendon, Willesden, and Walthamstow are exceptions to this rule.

In the large and the small towns having a high child mortality there is usually a high proportion of overcrowded tenements. It cannot be said, however, that the order in which these towns stand in respect of degree of overcrowding, as revealed by these particular statistics, is approximately the same as that of excessive child mortality; and Preston, Manchester, Rhondda, and Nottingham among the great

towns, and Stalybridge, Ashton-under-Lyne, Chorley, and Hyde among the smaller towns, have an excessive child mortality without a high proportion of overcrowding.

It will be noted that in Durham there is close association between dense crowding in houses and excessive child mortality. In this county housing conditions, speaking broadly, are deplorably bad. The standard of housing is low. A large proportion of the houses have only two bedrooms, often ventilating directly into the living room below. Scullery accommodation is defective. Often there is no pantry. Many streets and yards are unpaved. Drainage is defective, and privies and tub closets remain the chief sanitary conveniences instead of water closets. This general condition persists in large measure owing to the "free house" system; the houses under this system being owned by colliery proprietors. The Durham miners earn good wages, but they live in houses grossly inferior to the average workman's house in other areas, with terrible results in regard to the welfare of their wives and children. It is remarkable that the miners themselves do not appear to have taken up on any considerable scale the question of housing. It is important that more money should be spent on house rent in mining areas; and, so long as living under the conditions characterising present miners' houses continues, a large proportion of the wages spent in other directions must be regarded as misspent.

Similar remarks apply to other areas in which working men in the receipt of good wages themselves tolerate the continuance of bad housing. The margin of wages is spent in current pleasures and relaxation; and the associated occupancy of insanitary and inadequate houses means the continuance of unnecessary destruction of the health and lives of children.

More house pride, and a greater willingness to spend less on ephemeral pleasures and more on domestic comfort are needed. In short, elevation of the standard of living is an indispensable condition of progress. Already the concentration of public opinion in this direction is helping to bring this about.

Defective Sanitation.

The lack of exact proportion shewn in the tables (pp. 76, 77) between overcrowding and excessive child mortality is explicable by the varying extent to which overcrowding is associated with domestic uncleanliness, and with the retention of organic filth in and about the dwelling. Overcrowding, especially when there is also lack of cleanliness and of

PROPORTION PER CENT. OF POPULATION IN PRIVATE FAMILIES WHO LIVE IN A CONDITION OF OVERCROWDING—*i.e.*, IN TENEMENTS WITH MORE THAN TWO OCCUPANTS PER ROOM.

Large Towns.

Large towns with the twenty highest death-rates, 0–5

	Per cent. of over crowding (Census, 1911)	Birth rate, 1913
BURNLEY ...	9·5	22·8
WIGAN	12·9	28·1
MIDDLESBROUGH ...	13·4	31·1
ST HELENS	17·0	32·2
BARNSLEY	10·0	30·3
STOKE-ON-TRENT ...	8·6	31·3
LIVERPOOL	10·1	29·8
PRESTON	5·6	23·9
OLDHAM	7·2	23·0
SALFORD	10·1	27·1
WALSALL	7·2	30·0
WEST BROMWICH ...	12·2	29·5
MANCHESTER	7·2	25·7
ROTHERHAM	8·2	30·2
BOOTLE	9·2	30·0
GATESHEAD	33·7	29·2
SHEFFIELD	8·4	28·2
DUDLEY	15·0	28·6
SUNDERLAND	32·6	30·9
RHONDDA	5·6	33·1
NOTTINGHAM	4·3	22·7

Large towns with the twenty lowest death-rates, 0–5

	Per cent. of over crowding (Census, 1911)	Birth rate, 1913
Hornsey	3·2	16·2
Ilford	2·1	17·3
BOURNEMOUTH ...	1·6	15·6
Ealing	3·8	18·3
SOUTHEND-ON-SEA ...	3·6	18·5
HASTINGS	5·5	14·5
EASTBOURNE	4·3	16·0
BATH	4·8	15·7
OXFORD	2·4	17·7
READING	3·1	20·9
Swindon	2·2	23·5
EAST HAM	6·4	25·5
Walthamstow ...	7·4	24·4
Cambridge	2·3	19·5
Wimbledon	4·0	19·2
CROYDON	4·3	21·9
SOUTHPORT	3·5	15·2
Gillingham	2·3	22·5
Leyton	5·5	22·3
WALLASEY	3·3	22·1
Willesden	13·0	24·8

Smaller Towns.

Small towns with the twenty highest death-rates, 0–5

	Per cent. of over crowding (Census, 1911)	Birth rate, 1913
Ince in Makerfield ...	16·5	35·7
Stalybridge	5·0	23·7
Ashton under Lyne ...	4·9	23·2
Bilston	13·0	34·5
Leigh	8·0	27·9
Hindley	10·4	29·3
Farnworth	6·6	22·8
Widnes	12·6	31·9
Stanley	34·2	32·5
Chorley	5·3	23·7
Blaydon	41·2	32·8
Newcastle-under-Lyme	9·4	27·9
Tipton	16·8	34·2
Wednesbury	9·8	29·8
Hartlepool	28·3	32·3
Swinton and Pendlebury	7·9	23·8
Castleford	13·0	32·0
Oldbury	9·6	30·6
Tredegar	12·9	32·4
Hebburn	42·1	37·2
Ashington	32·2	37·1
Hyde	4·5	21·6

Small towns with the twenty lowest death-rates, 0–5

	Per cent. of over crowding (Census, 1911)	Birth rate, 1913
Southgate	1·7	18·8
Finchley	4·4	21·7
Tunbridge Wells ...	2·9	15·4
Reigate	3·0	15·8
Woking	3·8	19·9
Rugby	1·3	20·9
Weymouth	2·4	19·6
Sutton...	3·6	17·0
Sutton Coldfield ...	2·1	19·2
Beckenham	3·0	17·8
Bromley	3·3	17·5
Worthing	1·9	15·7
Hendon	8·8	25·6
Salisbury	1·2	20·9
Guildford	2·4	19·6
Harrogate	3·5	16·6
Barnes	4·7	22·0
Wood Green	5·1	22·2
Folkestone	3·0	18·3
Colchester	1·8	19·8
Watford	2·8	20·3
Winchester	2·6	17·6

Metropolis.

	Per cent. of overcrowding (Census, 1911)	Birth-rate, 1913
Shoreditch (241)*	36·5	31·5
Finsbury (216)	39·9	29·5
Bermondsey (201)	23·4	30·6
Bethnal Green (201) ...	33·2	30·7
Poplar (195)	20·6	31·9
Southwark (192)	25·9	30·8
Stepney (191)	35·0	29·2
Holborn (152)	25·6	16·6
St Pancras (151)	25·4	25·3
City of Westminster (131)...	12·9	14·1
Woolwich (128)	6·3	23·1
Chelsea (128)	14·9	19·1
Stoke Newington (121) ...	8·8	22·3
Lewisham (116)	4·0	20·4
Hampstead (112)	7·1	15·1

* The figures in brackets give the death-rates per 1000 births among children aged 0–5.

ventilation, implies chronic exposure to a stuffy dusty atmosphere, with excessive changes of temperature; it implies also, in most instances, that the food is stored under unsatisfactory conditions, and is often partially decomposed before being consumed. In tenement dwellings the storage of house refuse as well as of food in and close to the living room adds to the possibilities of mischief.

Domestic cleanliness is often rendered extremely difficult by the immediate surroundings of the dwelling. The yard may be unpaved or imperfectly paved. The sanitary conveniences, whether privies, pails, or water-closets, may be in an unsatisfactory condition, and give off noxious effluvia. From these sources, or from the yard into which the slop water may have been thrown, organic filth is trodden into the house.

Similar domestic contamination may come from unpaved or unscavenged streets, or from streets only imperfectly scavenged. Household refuse in some cases has to be taken through the house or even through the living room to the scavenger's cart. Infective material may be blown into the house, or may be brought in on shoes or boots or skirts, or carried in by flies bred in fixed ashpits or in other accumulations of refuse.

This statement illustrates the conditions of domestic infection to which the child, and especially the bottle-fed infant, is often exposed. The one condition common to all forms of domestic and municipal

insanitation is the risk to the child of inhalation or swallowing of harmful organic matter. It is not difficult to understand how these evils may occur in various insanitary circumstances, *e.g.*, when there is a foul privy or pail closet, an unpaved yard liable to contamination by slop water and other organic matter, or in a third storey tenement the occupier of which has to store house-refuse on the landing, and has no water supply or sanitary conveniences nearer than the ground floor.

Overcrowding on area and in dwellings, an excessive proportion of tenemented dwellings, and the associated difficulties in securing the elementary necessities of a cleanly and sanitary life, explain the exceptionally unfavourable position of Shoreditch among the metropolitan boroughs. Poverty is, of course, responsible for some of these conditions, but the converse is true, perhaps to an even greater extent, because of the readiness with which these conditions breed disease. If the conditions were ameliorated, the evils of poverty would be reduced. Much remains to be done in this and other metropolitan boroughs to improve the sanitation of tenemented dwellings.

In London, as elsewhere, anomalies occur in the relation between overcrowding and child mortality, as may be seen by reference to the table on p. 77. As a rule, however, the boroughs showing the highest degree of overcrowding have the highest child mortality. The anomalies are in part explicable by the fact that in some boroughs the population is more widely spread out, and a relatively larger proportion of the tenements with less than four rooms are self-contained houses.

The following seven metropolitan boroughs have the highest child mortality in London:

	Deaths under 5 per 1000 births	Proportion per cent. of tenements with less than 4 rooms	Proportion per cent. of overcrowded tenements
Shoreditch	241	73·6	36·5
Finsbury	216	80·8	39·9
Bermondsey	201	61·2	23·4
Bethnal Green	201	70·5	33·2
Poplar	195	58·2	20·6
Southwark	192	71·1	25·9
Stepney	191	67·5	35·0

The prevalence of diarrhoeal diseases is closely related to defective housing and to insanitation.

Industrial Employment of Married Women.

As stated previously in the report on Lancashire (p. 19), it is reasonable to believe that the industrial occupation of women, in so far as it exposes the pregnant mother to laborious work and strain, and in so far as it separates the infant from its mother, thus not only preventing suckling but also diminishing the individual care which the mother can devote to her infant, must tend to increase infantile sickness and mortality. In the textile districts industrial occupation of expectant or nursing mothers is seldom rendered necessary by poverty.

It would appear that the earlier children of such mothers are "minded" by a neighbour or some other person who undertakes this work for payment, until, as the family increases, the economic balance is altered and it becomes more profitable for the mother to stop at home than to go to the mill.

In a wider sense all industrial occupation of women, whether married or unmarried, may be regarded as to some extent inimical to home-making and child care. This is so, even in the case of girls, and it is important therefore that their industrial employment should be associated with systematic training in domestic economy.

This cause of excessive child mortality has been considered in preceding reports to the Board on child mortality.

In the first of these reports it was shewn that when the statistics of large communities are considered, the effects of the industrial occupation of women are concealed by the preponderant action of other adverse influences; a result not surprising in view of the fact that these latter influences affect either the entire population or a large portion of it, while usually a smaller section of the maternal population and their infants is affected by the industrial occupation of married women. The more general conditions affecting injuriously the welfare of young children are lack of medical care and nursing, defective housing (including deficient domestic food storage and uncleanliness), defects of domestic and municipal sanitation, crowding of persons on area, and carelessness or neglect of mothers, induced often by alcoholism or by overwork. These have already been considered.

That other evil conditions preponderate over the industrial occupation of married women as influencing child mortality is shewn in the statistics contained in this report. This may be illustrated by the experience of the towns enumerated in parallel columns below, the figures giving the total death-rate for each town at ages 0–5.

TOWNS WITH VERY EXCESSIVE CHILD MORTALITY.

(a) *Towns with a high percentage of extra-domestic occupation of married women.*		(b) *Towns with little extra domestic occupation of married women.*	
BURNLEY	257	Ince-in-Makerfield	288
WIGAN	254	MIDDLESBROUGH	251
Ashton-under-Lyne	247	ST HELENS	242
Farnworth	235	BARNSLEY	241
Chorley	229	STOKE-ON-TRENT	239
PRESTON	225	Bilston	237
OLDHAM	223	Widnes	231
SALFORD	219	Newcastle-under-Lyme	224
MANCHESTER	214	Wednesbury	221
Batley	208	Hartlepool	217
Heywood	205	WALSALL	216
BLACKBURN	202	WEST BROMWICH	215
LEEDS	202	ROTHERHAM	213
BURY	200	Oldbury	212
BOLTON	200	Tredegar	211
BIRMINGHAM	200	GATESHEAD	211
		SHEFFIELD	209
		DUDLEY	209
		Rhondda	207
		SUNDERLAND	207
		Ashton-in-Makerfield	202
		Stockton-on Tees	201
		MERTHYR TYDFIL	200
		Ebbw Vale	200

It will be seen that, although child mortality is very excessive in many textile towns in which there is a high proportion of industrial occupation of married women, it is even more excessive in some towns in which married women are seldom employed industrially. Several inferences may be drawn from these facts.

First. Among these inferences it is not justifiable to state that the industrial occupation of married women is not inimical to the health and welfare of their children. Cases in which a mother engaged in extra-domestic employment can provide adequate substitutional care for her children are exceptional. Such care cannot prove adequate

for an infant during the period of suckling, and industrial employment during pregnancy involves risks both to mother and infant. Except during periods of unusual industrial activity women generally remain at home until their infants are six months old. Hence the industrial employment of the mother affects particularly the health of the second half of infancy and of young children between 1 and 5 years old.

It may occasionally happen also that under circumstances of extreme poverty the money earned by the mother, who has to leave her infant for this purpose, may have greater influence in reducing infant mortality than the mother would be able to exercise under the circumstances of still deeper poverty which her stay at home would have meant. It will be agreed, however, by all, that under such circumstances the industrial employment of mothers is a serious evil, though it may be the lesser of two evils, the other being partial starvation for mother and infant.

Second. It being accepted that the industrial employment of the mothers of young children is, as a rule, mischievous, it may be inferred that the sanitary and social influences, apart from industrial occupation which endanger child life, are somewhat more numerous or more serious in such towns as Ince-in-Makerfield (288), and Middlesbrough (251), than they are in Burnley (257), in which there is the added danger associated with a large amount of industrial employment of women.

It may similarly be inferred that in such towns as St Helens (241), Stoke-on-Trent (239), Bilston (237), and Widnes (231) as well as in the large number of mining districts and of districts in which chemical industries, pottery works, and iron and steel manufactures are carried on, sanitary and social conditions, apart from industrial employment of mothers, are inferior to those of many of the textile towns which have an approximately equal child death-rate.

Third. There are great variations in child mortality in the towns in which there is large industrial employment of married women. This again appears to support the conclusion that the industrial employment of married women in numerous instances is a less potent factor in producing a heavy child mortality than other sanitary and social circumstances adversely affecting child life.

Size of Family in relation to Child Mortality.

That there is a common association of a relatively low birth-rate and a relatively low rate of infant mortality is shewn by our national statistics; and a corresponding association between high birth-rates

and high rates of infant mortality is frequently seen. Much more exact data on this important point will be available when the elaborate statistics as to fertility in different social strata, prepared in the General Register Office, are published. Meanwhile, there are available certain figures, published in the Registrar-General's annual report for 1911, which give valuable information. The following are illustrations of these statistics. The birth-rates are stated per 1000 married men in each social group:

	Birth-rate	Death-rate under one year per 1000 Births
Earthenware workers	84	172
Miners	107	160
Textile workers	50	148
Medical practitioners	52	39

On the strength of these and similar figures Dr Stevenson comments as follows: "The educated and comfortable classes have few children, of whom, under the favourable conditions provided, few die: unskilled labour produces many children, and loses a large proportion of them."

During recent years the decline of the birth-rate and the decline of child mortality have proceeded almost *pari passu*. This is shewn in the following table. Under each heading the relative rate for the period 1871–75 is given as 100:

	1871 to 1875	1876 to 1880	1881 to 1885	1886 to 1890	1891 to 1895	1895 to 1900	1901 to 1905	1906 to 1910	1911 to 1914
Legitimate births per 1000 married women, 15–45	294·6 = 100	100	95	91	88	83	78	72	66
Death rate under 5 years per 1000 of population 0–5	64·9 = 100	96	87	88	89	89	77	64	58
Death rate under one year per 1000 births	153 = 100	95	91	95	99	102	91	77	72

The question as to what is the character of the relationship between these fairly correspondent events has been mentioned in previous reports, and there is room for much difference of opinion. In my first report it was noted that comparisons of single counties show striking differences between the height of the infant death rate and of the birth rate. Thus, although in Durham, Glamorgan, and Northumberland both birth rate and infant death rate were in excess to about the same extent, the birth rate in the West Riding was 5 per cent. below and its infant death rate 8 per cent. above the average, the birth rate of Lancashire was 1 per cent. below and its infant death rate 22 per cent. above the average.

The figures in the present report bearing on this point are interesting. In a large number of towns, as shewn below, there is coincidence of relatively high birth-rate and relatively high child mortality at ages 0–5.

HIGH BIRTH-RATES AND HIGH CHILD DEATH-RATES.

	Birth-rate
Ince-in-Makerfield (288)	35·7
Bilston (237)	34·5
Tipton (222)	34·2
Rhondda (207)	33·1
ST HELENS (242)	32·2
Poplar (195)	31·9
Shoreditch (241)	31·5
Stoke-on-Trent (239)	31·3
SUNDERLAND (207)	30·9
Southwark (192)	30·8

The experience of the following towns shews that a high child mortality may be associated with a low birth-rate:

LOW BIRTH-RATES AND HIGH CHILD MORTALITY.

	Birth-rate
BOLTON (200)	21·8
BLACKBURN (202)	21·8
NOTTINGHAM (206)	22·7
BURNLEY (257)	22·8
OLDHAM (223)	23·0
LEEDS (202)	23·2
PRESTON (225)	23·9

The coincidence of low birth-rate and of low child death-rate is very usual, as may be seen by reference to the tables on p. 76.

There are no illustrations of a very high birth-rate and a low child death-rate among the towns enumerated in the appendices to this report. The most striking instance of a very low infant mortality with a very high birth-rate, when corrected for age distribution and marital condition, is furnished by Ireland, but this is secured under circumstances of life which are chiefly rural.

On a review of all the circumstances it does not appear necessary to alter materially the conclusion stated in previous reports, that the

connection often observed between a high birth-rate and a high rate of child mortality is probably due in great part to the fact that large families occur chiefly among the poorest classes, who are specially exposed to the influences producing excessive child mortality, while small families occur chiefly among the well-to-do, who might reasonably be expected to experience low rate of child mortality even if their families were larger.

Two further remarks should be made. If a large family implies such a degree of poverty as to produce deficient nutrition of mother or child, the child's prospects of health and life must be reduced. Short of this extreme poverty, it is evident that if a large family implies maternal overwork and insufficient attention to domestic cleanliness and to personal hygiene generally, the same result will be favoured.

Notwithstanding the general coincidence between declining birth-rate and declining child mortality in recent years, the numerous exceptions to this association quoted above do not permit of the conclusion that restriction of the birth-rate should play a part in the prevention of excessive child mortality. There is no reason to doubt that in the residential towns and suburbs, for instance (see table on p. 76), in which the association between low birth-rate and low child mortality is most obvious, larger families, which are nationally desirable, would be associated with a continuance of relatively low child mortality.

In recent years there has been a rapidly increasing intentional restriction of the size of families; and, as the standard of comfort of the population advances, the tendency in this direction becomes accentuated. There appears to be little prospect of abatement of this process. It appears likely that ere many years have passed the voluntary restriction of families will be the common practice in nearly all social grades, unless it becomes economically profitable to have large families, or unless steps are taken to diminish the expenditure involved by large families. Among the most important directions in which additional provision is needed are a skilled midwifery service readily available, adequate maternity nursing, and the provision of nursing and other assistance when the mother's solitary efforts are unequal to the domestic task.

II. SUMMARY OF ACTIVITIES IN MATERNITY AND CHILD WELFARE WORK.

For the convenience of workers concerned with the conservation of the life and health of mothers and their young children a catalogue of present activities and of further activities on the part of local authorities and others, which would conduce to this end, is here given.

The activities particularly concerned may be divided into four groups:

1. Housing.
2. Intra-domiciliary and extra-domiciliary sanitation.
3. Food supplies.
4. Medical assistance, including nursing.

The first three of these need not be set out in full.

Housing.

No family can be regarded as housed under conditions which fulfil the needs of health unless the house or tenement provides adequate sleeping accommodation, and comes up to the following minimum standard in other respects:

1. An adequate kitchen and living room, possibly the two combined.
2. Cool and dustless storage for food.
3. A scullery with sink and water supply within the dwelling.
4. Satisfactory storage for coal and a movable covered ashbin.
5. Separate sanitary conveniences for each family.

Cleanliness and avoidance of food contamination cannot reasonably be expected unless these conditions are fulfilled; and the list of requirements here set out cannot be regarded as completing what is desirable.

Sanitation.

1. In towns the closet should be a water-closet.
2. The back yard, or at least the portion abutting on the house, and a path leading to the street, should consist of impervious material.
3. Street scavenging must be satisfactory.
4. Every aid to domestic cleanliness as by accessible water supply and ready disposal of "slops," must be available.

In this connection the importance of the use of overalls and of the provision of baths for the cleansing of workers, *e.g.*, of miners, before they return from their work, needs much greater attention than it has hitherto received.

Food Supplies.

Aids to the satisfactory storage of food after it reaches the home have been indicated above. Precautions against access of dust and flies to milk should be universally adopted.

The preparation of food under proper conditions depends largely on the provision of satisfactory cupboards and food stores, and on ready access to water supply.

The provision of a pure milk supply is largely out of the control of the individual householder; in regard to this, as well as to many of the items of housing and sanitation enumerated above, the local authority cannot divest itself of serious responsibility.

Until or unless it can be guaranteed that cow's milk is derived from cows which have been proved to be free from tuberculosis it is important that mothers should be advised to boil all milk before it is given to infants and young children.

Medical Assistance, including Nursing.

Fuller detail is given under this heading, as it is the part of maternity and child welfare work which in the past has received least attention, and as it is the part of this work in which under present circumstances there are the greatest possibilities of saving life and of preventing illness and disablement.

It has been stated in a previous chapter that in degree of ignorance there is little if any difference between the wives of wage-earners and the wives of men belonging to other classes. *The difference, apart from the handicap of the former in respect of housing, food supply, and sanitation, in the main is one of ability to secure the assistance required in the various contingencies of maternity and early childhood.*

What is the assistance required, and how can the local authority and their officers become advised of the need?

In order that assistance may be available it is necessary that the officers of the local authority and the mother should be brought into relation with each other.

The chief means for this are furnished under the Midwives Act and the Notification of Births Acts.

Midwives Act.

The Midwives Act regulates the practice of midwives who attend more than half of the total confinements in England and Wales, these being, as a rule, the confinements in which the additional medical and nursing assistance considered under this section are most needed.

The local supervising authorities under the Midwives Act are the county borough councils, the county councils, and those councils within county areas to whom county councils have delegated their functions under this Act.

It is the duty of the local supervising authority—

(*a*) To exercise general supervision in accordance with the rules of the Central Midwives Board over all midwives practising in their area;

(*b*) To investigate charges of malpractice, negligence, or misconduct, on the part of any midwife practising within their area, and, if a *primâ facie* case is established, to report the same to the Central Midwives Board;

(*c*) To suspend any midwife from practice, in accordance with the rules of the Central Midwives Board, if this appears necessary to prevent the spread of infection;

(*d*) To report at once to the Central Midwives Board the name of any midwife practising in their area convicted of any offence;

(*e*) To keep a roll of midwives practising in their area, and to report the death or change of address of any midwife to the Central Midwives Board;

(*f*) So far as practicable, to give due notice of the effect of the Act to persons at present using the title of midwife.

It is evident that the above duties, if fully carried out, have most important bearing on the prevention of mortality and on the diminution of suffering in child-bearing, as well as on the prevention of infant mortality. Much information as to the administration under the Midwives Act will be found in the annual reports of the Central Midwives Board, on pp. 137–148 of the *Report on Infant Mortality in Lancashire* (Cd. 7511), and on pp. 60–104 of the *Report on Mortality in connection with Childbearing* (Cd. 8085). There is large scope for more exact and detailed work in many administrative areas.

Rules of Central Midwives Board for Midwives.

The individual midwife has most important duties in relation to maternity and child welfare schemes, and more generally to the work of local supervising authorities, and the part which she can take in this work will steadily increase as time goes on.

Training of midwives. An important step forward has been taken by the authorisation by the Privy Council of an extension of the period of training required before a woman can be examined by the Central Midwives Board with a view to her obtaining a certificate of qualification for practice. The period of training must now extend over a period of not less than six months; certain classes of nurses, however, only being required to undergo four months' special training.

The new rules framed by the Central Midwives Board, and approved by the Privy Council on 23rd June, 1916, contain important directions for midwives bearing on maternity and child welfare work.

It is convenient to summarise here the new rules, so far as they relate to this subject.

Ante-natal work of the midwife. In the first rule (E. 1) it is stated that—

> When engaged to attend a labour the midwife must interview her patient at the earliest opportunity to inquire as to the course of the previous pregnancies, confinements, and puerperia, both as regards mother and child, and to advise as to personal and general arrangements for the confinement, and, with the consent of the patient, visit the house.

Register. By Rule 24 the midwife is required to keep a register in the following form:

No.
Date of expected confinement.
Name and address of patient.
Age.
Number of previous labours and miscarriages.
Date and hour of midwife's arrival.
Presentation.
Date and HOUR of child's birth.
Sex of infant. Born living or dead.
Full time or premature. Number of weeks.
Name of doctor, if called.

Complications (if any) during or after labour.
DATE OF MIDWIFE'S LAST VISIT.
Condition of mother then.
Condition of child then.
Remarks[1].

This register comprises a statement of any previous labours or miscarriages of the patient, and can thus be made the basis of valuable instruction to the midwife by the inspector of midwives as to the circumstances in which she should recommend patients to seek medical advice.

This principle is accentuated by the following note, immediately preceding Rule 1, which, although not stated in an obligatory form, must in the future have great influence in leading midwives to secure medical advice for their patients as required. The note is as follows:

> NOTE. Whenever illness or abnormality has occurred in the previous pregnancy, and whenever the previous pregnancy has ended in an abortion, a premature labour, or a still-birth, the midwife, on being engaged to attend the patient in her next confinement, should explain that the case is one in which skilled medical advice is required, and should urge the patient to seek advice from her medical attendant, or at a hospital or other suitable institution.

From the present point of view Rule 20 is also important:

> 20. In all cases of illness of the patient or child, or of any abnormality occurring during pregnancy, labour, or lying-in, a midwife, as soon as she becomes aware thereof, must explain that the case is one in which the attendance of a registered medical practitioner is required, and must hand to the husband or the nearest relative or friend present the form of sending for medical help (see Rule 23 (*a*)), properly filled up and signed by her, in order that this may be immediately forwarded to the medical practitioner or approved institution.

So far as pregnancy is concerned, this rule particularly applies to the following specified conditions:

Deformity or stunted growth.
Loss of blood.

[1] If any drug, other than a simple aperient, has been administered in any way, state here the name and dose of the drug, and the time and cause of its administration. (See Rule 19.)

Abortion or threatened abortion.
Excessive sickness.
Puffiness of hands or face.
Fits or convulsions.
Dangerous varicose veins.
Purulent discharge.
Sores of the genitals.

Notification of Births.

The notification of births within thirty-six hours enables visits to be made on behalf of the Public Health Authority as early after notification as is thought necessary. Whether the first visit need be made during the time when the midwife is in charge of the patient will be decided by the medical officer of health in the light of his knowledge of the particular circumstances of each case. As stated in my *Memorandum on Health Visiting*, etc., an immediate visit after notification will become less generally necessary "when all midwives are prepared to give the best advice to the mother respecting the management of her infant." It is indispensable for successful co-operation that a friendly relationship should exist between the midwife and the health visitor, and in bringing this about the superintendent of midwives, if not the same person as the health visitor, can be most helpful both in regard to individual cases and by arranging opportunities for collective discussion of difficult points.

Rule 12 of the Central Midwives Board makes the midwife—

> Responsible for the cleanliness, and for giving all necessary directions for securing the comfort and proper dieting of the mother and child during the lying-in period, *i.e.*, normally for ten days after the labour;

and the following important note is added to this rule:

> NOTE. The midwife should endeavour to promote breast feeding, and should, when breast feeding cannot apparently be continued, urge medical advice.
>
> Many Public Health Authorities now provide health visitors, maternity centres, or baby welcomes for the assistance of mother and child. It is desirable that the midwife when she ceases attendance should advise the patient to avail herself of such help.

Notification of still births. Under the Notification of Births Acts, still births after the twenty-eighth week of pregnancy are required to

be notified to the medical officer of health. Midwives are required under Rule 22 of the Central Midwives Board to notify still-births to the local supervising authorities in all cases where a registered medical practitioner is not in attendance at the time of birth. In regard to midwives there is no limitation of this duty to still-births occurring in the latter part of pregnancy, and the form of notification prescribed by the Central Midwives Board requires that the month of pregnancy, and the condition of the child (whether macerated or not), shall be stated.

Already much valuable inquiry is being made by some local authorities into the circumstances under which still-births have occurred; and by bringing these cases into relation with the work of the maternity centre or, apart from this, by securing examination of pathological material, there is a good prospect of action which will secure improvement in the health of mothers and secure the live-birth of a larger number of infants.

It is scarcely necessary to summarise the duties of the midwife in regard to the mother and child during the lying-in period, as set out in the rules of the Central Midwives Board, though these evidently have important bearing on maternity and child welfare work.

The various forms of public medical and nursing work in connection with maternity and child welfare, and the conditions calling for them, may now be enumerated.

I. PRE-MATERNITY WORK.

This includes arrangements as to

- **Hygiene of the mother*, especially as to feeding, condition of teeth, fatigue, possibilities of infection, conditions of housing;
- **Instruction of the mother*, as by health visitors and at maternity centres;
- *Feeding of the mother* when required by voluntary funds;
- **Treatment of the mother* at the maternity centre or in a pre-maternity ward of a hospital, provided or contracted for by the local authority. This may be required for many minor ailments, the treatment of which will improve the health

* All the items marked with an asterisk come within the scope of the Regulations of the Local Government Board as to grants for Maternity and Child Welfare; and grants covering one-half of the total expenditure will be made in respect of approved expenditure under these headings for work carried on to the satisfaction of the Board.

prospects of the mother and her infant. On these, see p. 23 of *Memorandum on Health Visiting*.

The treatment of dental caries and of oral sepsis comes within the scope of this work.

Treatment may also be required *inter alia* for the conditions enumerated on p. 91, which the midwife has to notify to the local supervising authority. When such notification takes place there should be inquiry by the local supervising authority, and the necessary steps should be taken to ensure the requisite medical assistance and nursing.

An important form of preventive treatment consists in periodical examination of urine, with a view to averting eclampsia.

The pre-maternity ward of, or beds in, a hospital should be available for major complications of pregnancy, *e.g.*, vomiting of pregnancy, haemorrhages, eclampsia, and for conditions requiring Caesarean section or the induction of premature labour.

**Treatment of the mother for special diseases.* See under XI. and XII.

Notification of pregnancy. This is not advised, except under the restricted conditions stated on p. 22 of the *Memorandum on Health Visiting*, etc.

II. Still-births and Abortions.

Information as to these is received by notification. Judicious inquiry made through the private practitioner, or very carefully when there is no private doctor, will be valuable for the mother.

*(*a*) Examination of products of pregnancy may show spirochaetes of syphilis. (See under XII.)

*(*b*) Examination of maternal blood may give a positive result to the Wassermann test. (See under XII.)

Examinations into the incidence and the circumstances of still-birth may throw light on the competence of a particular midwife.

Occasionally, infants live born are returned as still born.

* Items marked thus form the subject of grants from the Local Government Board apart from Maternity and Child Welfare schemes. For approved expenditure on tuberculosis 50 per cent., and for approved expenditure on venereal diseases, 75 per cent. of the total expenditure is paid by the Board.

*The provision of a maternity nurse for cases of abortion in the patient's own home is very important in obviating subsequent disablement of the patient.

*The provision of hospital treatment for some of these cases is also greatly needed.

III. CHILDBIRTH.

It is important to ascertain in each area that an adequate service of midwives is available.

*If this is not so, steps should be taken by the council directly or through a nursing association, as explained in the Board's circular of 23rd September, 1916, to supplement this service.

*The local authority may with the Board's sanction, under Sec. 133 of the Public Health Act, 1875, provide a midwife and a doctor when necessary for necessitous women in their confinements. When this has been done midwives and doctors should be informed of the arrangements.

*The provision of hospital beds for women in complicated childbirth is most important. A great increase of this accommodation would greatly reduce mortality in childbirth and disablement after childbirth. Grants are available for this purpose when the beds are provided or contracted for by the local authority.

IV. THE LYING-IN PERIOD. THE MOTHER.

*It should be made known that the local authority can provide a qualified maternity nurse in necessitous cases by the local authority or by a voluntary society, *e.g.*, a nursing association.

For the mother confined at home, voluntary assistance by means of societies providing "home helps" is most valuable. At present this help is largely given by neighbours, but it is commonly inadequate to secure sufficient rest and freedom from anxiety for the mother.

*The local supervising authority should, through the medical officer of health and the inspector of midwives, endeavour to get into personal touch with midwives while they are in attendance after confinements in a much larger proportion of cases than is now commonly done.

*Hospital treatment may be required during this period for certain complications.

*This treatment is particularly important for a large proportion of cases of puerperal pelvic infection (puerperal fever). It may be given

* See footnote on page 92.

at the isolation hospital, or in a general or women's hospital. It is important always to have one or more beds available for this purpose.

Careful investigation of the source of each case of pelvic infection is required on the part of the medical officer of health. This should be undertaken in co-operation with the doctor or midwife in attendance.

* Attention is drawn to the case mortality of puerperal fever [see p. 27 of *Report on Maternal Mortality in connection with Child-bearing* (Cd. 8085)], which varies greatly in different areas. Persistent efforts should be made to establish such arrangements with medical practitioners as will ensure notification of each case of puerperal infection.

V. The Lying-in Period. The Infant.

A special risk to the infant during this period is ophthalmia neonatorum. The rules of the Central Midwives Board require that information should be sent to local supervising authorities as to "inflammation of, or discharge from, the eyes, however slight." They also require the midwife to hand to the husband or the nearest relative or friend the form of sending for medical help [Rule 23 (*a*)]. Every medical practitioner called in to a case of ophthalmia neonatorum which has not been previously notified to the local medical officer of health is required to notify it. Unless the case has already been notified by a medical practitioner it is the duty of the midwife to do so.

* It is important that the local authority should offer facilities for prompt bacteriological examination of pus from the eyes of infants. (See under XII.)

* Also that they should when needed provide nurses for these cases under medical supervision. Prompt action on these lines is necessary to save eyesight.

* If this treatment cannot be carried out effectively at home the infant and the mother should both be removed to a hospital. Grants are available for such hospital treatment when provided or contracted for by the local authority.

In some areas one out of every 25 infants born die in the first week after birth, and in other areas only one out of every 60 born die in the first week of extra-uterine life [see p. 27, *Second Report on Infant Mortality* (Cd. 6909)]. Evidently there are unfavourable circumstances not solely ante-natal, but in large measure natal and post-natal, which

* See footnote on page 92.

medical and hygienic supervision during this period might reduce. Local investigation of this excessive mortality in the first week (and in the first month) of life is greatly needed.

VI. THE NURSING MOTHER.

Much illness and disability is due to the nursing mother not having adequate assistance in domestic work. Co-operative effort should do much to diminish this difficulty by a system of home helps working from house to house. The mother often cannot successfully cope with the needs of a large family and of an infant, especially if the infant has to be artificially fed.

*Under the Local Government Board's regulations in aid of maternity and child welfare work, aid is offered for a centre providing "medical supervision and advice for expectant and nursing mothers, and for infants and young children, and medical treatment at the centre for cases needing it."

*Similar provision is made for "hospital treatment, provided or contracted for by a local authority, for complicated cases of confinement or complications arising after parturition, either in the mother or infant, and for infants found to need in-patient treatment."

*When disability of nursing mothers results from parturition hospital treatment can be provided under the Board's regulations.

Good work is being done by voluntary agencies in providing meals, or a daily supply of milk for nursing as well as for expectant mothers.

VII. THE INFANT AND CHILD TO THE AGE OF 5.

*The most important first task is the appointment of a staff of health visitors who will be sufficient in number to visit each infant at intervals during infancy, and keep in touch with the child and its mother by home visiting and by attendance at a child welfare centre until school attendance begins.

As already indicated, health visitors are needed for expectant and nursing mothers as well as for young children. There is much in favour of this work being carried out throughout by the same visitor, but local circumstances and the qualifications of the visitor will need to be considered in deciding whether this is to be the case.

The health visitor may also be tuberculosis nurse or school nurse.

* See footnote on page 92.

In scattered areas it may be desirable to appoint the district nurse as health visitor.

*Infant Consultations, including medical treatment for children requiring it, should be provided. At these Consultations all the children should at intervals be kept under medical supervision.

*The treatment given at these child welfare centres may include the treatment of such conditions as adenoids, dental caries, etc.

For these purposes a combination may be able to be arranged between the work of the child welfare centre and the school clinic.

VIII. The Supervision of Illegitimate Children, etc.

Illegitimate births formed 4·2 per cent. of the total births in England and Wales in 1914, and the death-rate of illegitimate infants is twice as high as that of legitimate infants.

There is great need for increased supervision of the welfare of illegitimate children.

The aim should be, whenever practicable, to prevent the separation of the mother from her infant during the first year after birth. This has important moral value as well as value in securing continued parental care. There is large scope for increased voluntary work in this connection.

Institutions for the reception of infants, especially of illegitimate infants, generally experience a very heavy death-rate. A system of home visiting of the mothers or foster-mothers, adequately supervised, in most instances is preferable to such institutions.

The Children Act outside the metropolis is administered by the Boards of Guardians. If the inspectors under this Act are not also the health visitors of the local authority, their work should be carried on in close co-operation with the latter.

IX. Relation to General Sanitary Work.

During pregnancy it is important that the condition of the home as to cleanliness within and about the house, and as to overcrowding, as well as preparations for childbirth, should be made satisfactory. During childbirth, and for children under five the importance of domestic sanitation can scarcely be exaggerated.

To aid in securing normal childbearing and healthy childhood it is important that each of the items enumerated on pp. 85 to 91,

* See footnote on p. 95.

should be satisfactory. This work is amongst the most important duties of the health visitor, and her report to the medical officer of health should always include a statement on the above items.

X. Relation to Educational Work.

Collective instruction of mothers is necessarily much less useful than individual counsel directed to the needs of the individual mother or child. But at centres at which mothers attend, teaching in the elements of hygiene, in cooking, dressmaking, and domestic economy forms a valuable auxiliary to the more essential branches of maternity and child welfare work.

XI. Tuberculosis.

*In special cases, mothers and their children should be referred to the tuberculosis officer for special treatment.

In such cases the official machinery for inquiring into home conditions and for examination of "contacts" should be utilised.

XII. Venereal Diseases.

*Syphilis is a common cause of abortion and miscarriage; hence the importance of utilising the facilities provided under official schemes for the treatment of these diseases for:

(*a*) Clinical examination of patients.
(*b*) Confirmatory diagnosis by examination of foetal material or by the Wassermann test.
(*c*) Treatment of patients.

Syphilis is a common cause of malnutrition and disease in infancy and childhood, and when such evidences of this disease as mucous tubercles or interstitial keratitis are found, the patients should be referred to the special treatment centre.

When the confidence of the mother has been secured, an effort should be made to have other members of the family examined with a view to their treatment if this is found to be necessary.

*Gonorrhoea in the mother may cause ophthalmia neonatorum in the infant, which is considered in paragraph V. The condition of the mother should also receive attention.

* See footnote on page 91.

XIII. Measles.

The Local Government Board's regulations as to measles open up new possibilities of diminishing the heavy loss of child life from this disease.

*The visitation of cases of measles should be undertaken, when practicable, by health visitors.

*The further care of selected cases of measles by providing, when necessary, medical attendance and nurses, forms an important part of child welfare work.

Hospital provision for selected cases is also of great value.

XIV. Whooping-cough.

In the Board's circular letter of 31st March, 1915, the offer is made to enable any sanitary authority to secure the notification of this disease, on the lines of the Measles Regulations.

*Health visitors can undertake valuable work in securing precautions against infection and against serious complications.

For both measles and whooping-cough there is need for organisation of supervision during convalescence.

Convalescent homes for children who have recently recovered from these diseases would be of immense benefit in avoiding deafness, and in decreasing the likelihood of subsequent development of tuberculosis.

In visiting cases of measles and whooping-cough the risks of unboiled milk in causing tuberculosis should be explained.

XV. Diarrhoeal Diseases.

*The work of health visitors and of child welfare centres should greatly diminish these diseases.

Each June and July, before the diarrhoeal season begins, a special campaign should be organised to minimise diarrhoea. This will be (*a*) general, (*b*) special and individual. The general measures are those of general sanitation and of protection of the milk supply. The individual measures should be directed specially to the children under two years old whose addresses are known from the Notification of Births Register and the subsequent visits of health visitors.

In some areas notification of cases of summer diarrhoea has been arranged, but so far the action taken in most of these areas has not

* See footnote on page 91.

been so complete as to prove the value of notification. Special visits in July to infants in the poorer streets are desirable. Attention should be concentrated especially on bottle-fed infants. These will have been previously noted in the records of the health visitor. Personal instruction to each parent is much more efficacious than the delivery of leaflets of advice or any form of theoretical instruction.

If the health visitor is in sympathetic touch with parents, voluntary information of the occurrence of diarrhoea is often given to her.

With the sanction of the Local Government Board medical and nursing assistance may be afforded.

*For some patients removal to hospital greatly improves their prospects of recovery. Grants are available for beds specially provided by the local authority for diarrhoeal patients sent from the child welfare centre.

* See footnote on page 91.

A NOTE ON THE LAG-PHASE IN THE GROWTH OF MICRO-ORGANISMS.

By ARTHUR SLATOR.

(With one Chart.)

The mechanics of the growth of micro-organisms in nutrient solutions has of late years received considerable attention. The logarithmic law governing the phase of unrestricted growth is now well established (Lane-Claypon (1909), Penfold and Norris (1913), Slator (1913), (1916) and others). Considerable information regarding another period of growth is also available. When a suitable nutrient medium is seeded with bacteria, there is usually a period during which the bacteria grow at a slower rate than is the case later when the logarithmic law holds good. This period is called the lag-phase of growth. The laws governing such growths have been carefully and successfully worked out by Penfold (1914) and Ledingham and Penfold (1914). *Bacillus coli* was the organism employed in their experiments. In the paper by Ledingham and Penfold on "The mathematical analysis of the lag-phase in bacterial growth" the authors have shown that the relationship between the number of bacteria and the time can be represented by an equation involving two constants both of which vary in different experiments, but remain of the same value throughout each single experiment. It has apparently escaped notice that there is a relationship between the two constants of such a nature that one of them can be replaced by a third which remains of the same value throughout the whole series of experiments. If use is made of this new constant further information regarding the lag-phase in growth can be obtained.

The notation employed in this paper is essentially the same as that used by Ledingham and Penfold. Log = logarithm to base 10, *ln* = natural logarithm to base *e*, G.T. = generation time.

Ledingham and Penfold show that the growth of bacteria during the lag-phase is accurately represented by the equation

$$X^n = k \log Y \text{ ....(1)},$$

where Y is the number of bacteria after a time X, the initial seeding being one. n and k are constants. They find the following values for n and k in eight separate experiments.

TABLE I.

Experiment	n	k	$\frac{k}{n}$	$\log \frac{k}{n}$	$\frac{\log k/n}{n}$
1	1·88	10988	5840	3·766	2·00
2	1·77	6322	3570	3·553	2·01
3	1·56	2329	1490	3·173	2·03
4	1·56	2465	1580	3·199	2·06
5	1·97	16732	8490	3·929	1·99
6	1·74	5483	3150	3·498	2·01
7	2·01	23020	11450	4·059	2·02
8	2·7	1045000	387000	5·588	2·07
					Average = 2·024 = A

It is clear from this table that there is a relationship between n and k of such a kind that $\frac{\log k/n}{n} = A$, a constant for the whole series of experiments.

Now let $\quad 10^A = \frac{1}{K_1}$, then $k = n10^{nA} = \frac{n}{K_1{}^n}$ ....(2).

Equation (1) then becomes

$$X^n = \frac{n}{K_1{}^n} \log Y \text{ or } X^n K_1{}^n = \log Y^n \text{ ....(3)}.$$

For this series of experiments A averages 2·024. Therefore

$$K_1 = 10^{-2 \cdot 024} = 0 \cdot 00945.$$

The equation of the lag-phase of growth reads therefore

$$X^n (0 \cdot 00945)^n = \log Y^n.$$

Differentiating equation (3) we have

$$nX^{n-1} K^n dX = \frac{n}{Y} \log e dY,$$

or

$$\frac{\frac{dY}{dX}}{Y} = \frac{K_1{}^n X^{n-1}}{0 \cdot 4343}.$$

This value $\frac{dY}{dX}/Y$ is the "constant" of growth at any time X and can be called Z.

Therefore $$0{\cdot}4343Z = K_1{}^n X^{n-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4).$$

The equations of unrestricted growth corresponding to (3) and (4) are

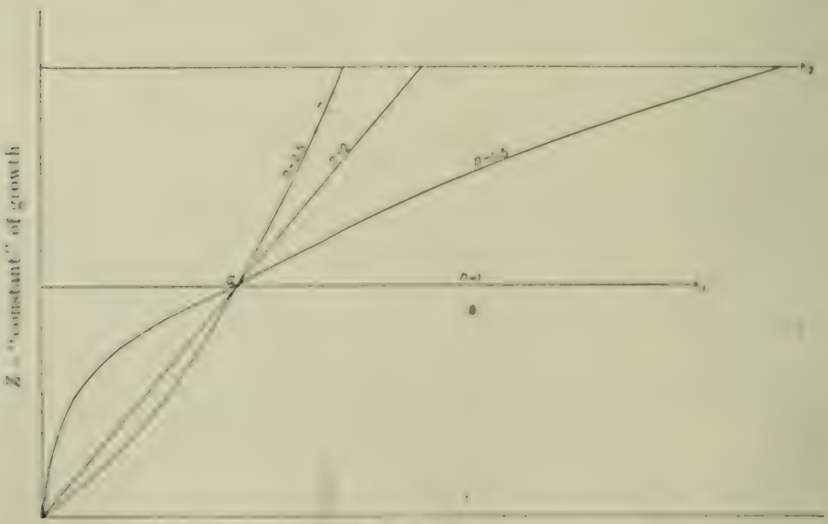
$$XK_2 = \log Y \quad \ldots\ldots\ldots\ldots\ldots\ldots (5).$$

$$0{\cdot}4343Z = K_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (6).$$

$\text{G.T.} = \frac{\log 2}{0{\cdot}4343Z}$ in both cases.

The minimum generation-time found by Ledingham and Penfold is usually 18–20 mins., though in some cases values 16·6 and 17·1 mins. have been observed (Experiments 5 and 8, pp. 253 et seq.). The lowest value 16·6 mins. corresponds to a value of $K_2 = 0{\cdot}0182$. K_2 is therefore approximately equal to $2K_1$. The constant $K_1 = 0{\cdot}00945$ corresponds to a G.T. of 32 mins.

The peculiarities of the lag-phase can be conveniently discussed by the aid of a $Z - X$ diagram. Four of the family of curves given by the general equation $0{\cdot}4343Z = K^n X^{n-1}$ are shown in the chart. They



are the particular cases when $n = 1$, $n = 1{\cdot}5$, $n = 2$ and $n = 2{\cdot}5$ and they illustrate the general shape of the curves with varying n. The following relationships are evident. All curves pass through a point a.

When $n = 2$ the curve is a straight line joining the origin to the point a. When $n = 1$ the curve is a straight line passing through a parallel to the time axis. When $n = 2{\cdot}5$ the curve rises slowly at first and then rapidly. When $n = 1{\cdot}5$ the curve rises rapidly and then slowly.

All these curves theoretically can be continued indefinitely but in practice they end abruptly when they cut the horizontal line corresponding to the minimum G.T., that is $0{\cdot}4343Z = K_2$. When $n = 1$ the lag is infinitely great. The equation of unrestricted growth is evidently a limiting case where $n = 1$, but the value of K is about twice as great as in the lag-phase ($K_2 = 2K_1$). If there exist growths of bacteria which exhibit values of n approaching 1 the minimum G.T. will be reached only after a long time, and growths will be obtained which give apparently constant values of G.T. higher than the true minimum value. An average value $K_1 = 0{\cdot}00945$ corresponds to a point $1/0{\cdot}00945$ mins. (106 mins.) from the beginning of the experiment. All growths therefore (no matter what n is) should give a G.T. 32 mins. at a time 106 mins. Ledingham and Penfold's figures, given on pp. 252 and 253, show this to be approximately the case. Closer agreement would be obtained if the figures were adjusted to an extrapolated time origin when $Z = 0$ (G.T. $= \infty$). Ledingham and Penfold's results show another peculiarity which can be pointed out in the following way. When yeast cells are placed in suitable sugar solution, the rate of fermentation is proportional to the number of yeast cells present. This result can be expressed by means of the equation $qt =$ constant, where q is the quantity of yeast and t the time to bring about a given amount of fermentation. Arrhenius, in his book, *Quantitative Laws in Biological Chemistry*, shows that this qt-rule holds good for a large number of reactions brought about by cells and enzymes. In the case of a reaction which takes place in the cell itself it is a result which would be anticipated and deviations from the qt-rule are usually more interesting than agreement with it. We have such a deviation in Ledingham and Penfold's results. If the qt-rule held good n would be constant when the medium and condition of the seeding were the same, independently of the amount of seeding. Thus the equation $X^nK^n = \log Y^n$ takes no note of the seeding only of ratios between the seeding and number of cells at time X. Ledingham and Penfold find however that n varies with the seeding. The probable explanation is that n is influenced by a substance present in small concentration in the medium and that the amount is not great compared with the seeding used.

A knowledge of the factors which influence n would no doubt help to determine the cause of lag and the physical meaning of n.

Possibly growth depends on a substance present in the cell and is at all times proportional to its concentration z. For the convenience of having a name for this substance it has been called the "enzyme of growth." If z were initially zero and increase with the time during the lag-phase according to the equation $z = at^b$ where a and b are constants ($b = n - 1$) an explanation of the lag-phase would be obtained.

No doubt both the medium in which development takes place and the condition of the cell play their part in determining n.

It is possible that cultures of micro-organisms can be obtained which grow in one medium but not in another, although they grow readily in the latter medium if care is taken to get rid of lag. Irregularities in the growth of yeast in certain nutrient solutions have given rise to the idea that a certain mysterious "Bios" is necessary for yeast growth. The experiments on which the idea of "Bios" is founded can be readily explained by peculiarities in the lag-phase of growth. Text books by Bayliss (1915) and by Sykes and Ling (1907) give accounts of these experiments.

It is difficult to understand why the relationship shown in Table I holds good only for logarithms to the base 10. If natural logarithms are used another constant has to be introduced which cannot be eliminated by giving K another value.

Using natural logarithms equations (3) and (4) read

$$X^n a K^n = ln\, Y^n \quad \text{.............................(7).}$$

$$Z = aK^n X^{n-1} \quad \text{.........................(8).}$$

There are certain advantages to be gained by using equation (8) as the fundamental equation of growth.

Let us consider the case when n is less than 1. By keeping K and a constant and by varying n we get a family of hyperbola-like curves all passing through a given point. The possibility that such curves represent rates of retarded growth of micro-organisms is worth investigating. When a culture of bacteria or yeast develop in a suitable medium after the logarithmic phase there occurs a period of retarded growth brought about by changes in the medium or by scarcity of food. The retarding influences finally become so great that growth ceases. The laws governing growth under such conditions are not easy to determine for the retarding factors do not remain constant and usually more than one is at work. It should however be possible to devise

experiments in which the retarding influence remains constant and to determine the $Z - X$ curve under these circumstances.

In the special case where $n = 0$ and $a = 1$, $ZX = 1$ or the $Z - X$ curve is a hyperbola.

Further $Z = \frac{dY}{dX}\Big/Y$. Therefore $\frac{dX}{X} = \frac{dY}{Y}$ or $Y = cX$, where c is a constant. The curve of growth is therefore a straight line.

The interest in this calculation lies in the fact that rectilinear curves of yeast growth have been observed by H. T. Brown (1914). Brown explains these curves on the assumption that yeast growth requires oxygen and that the lack of oxygen under the conditions of the experiments has a retarding effect on the growth just sufficient to change the logarithmic curve to a rectilinear one. There is no doubt that if oxygen acts in the manner he describes the $X - Y$ curve becomes a straight line. On the other hand if the influences retarding growth are such that no increase in the amount of "enzyme of growth" takes place then rectilinear curves of growth would be obtained, and there is no difficulty in explaining Brown's results on such lines.

Whatever the true explanation is, rectilinear curves of growth can be expected if equation (8) represents the growth when retarding influences come into play.

Another phase in the existence of growths of micro-organisms is the final one when they gradually die. The curve of disappearance of living cells has been shown to be logarithmic in character[1] and may

[1] Though the logarithmic curve of the dying of cells and organisms seems to be followed in a surprisingly large number of cases it is not difficult to find conditions under which such a law would not hold. Thus for instance the natural death rate of a number of men all of the same age is not of a logarithmic character. This is shown in Table II, where

Y = the number of men surviving at various ages. The figures are taken from a table on the "Expectation of Life" based on the mortality for the 10 years 1891—1900. (See *Whitaker's Almanack*, 1917, 452).

Z = "constant" of decrease and is calculated over a short period of time (1 year) at various ages. Z is assumed to be constant over this short period and is measured by the difference between the logarithms of Y at the beginning and end of the year.

Z increases rapidly with the age of the man. By subtracting a constant from Z we get a series of figures ($Z - 0{\cdot}0011$) which are approximately in geometrical progression. This is shown in the last column where A is calculated from the equation

$$A = \frac{1}{x} \log \frac{Z_0}{Z_x},$$

where $Z_0 = Z - 0{\cdot}0011$ at age 20 and $Z_x = Z - 0{\cdot}0011$ at age x.

The constancy of A shows that the decrease in the number can be calculated by means of an equation of the type $Z = a + e^{kx}$.

The death rate is apparently determined mainly by two factors, the one a constant

therefore be considered a special case of the curve given by the general equation.

The equation

$$Z = aK^nX^{n-1},$$

covers therefore the main phases of growth of micro-organisms developing in a nutrient medium. By giving n values greater than 1 and keeping a and K constant curves representing the lag-phase of growth are obtained. When $n = 1$ and a is suitably adjusted the logarithmic period of growth is obtained. When $n = 0$, $a = 1$, special rectilinear curves of retarded growth are obtained. When $n = 1$ and a is given a suitable negative value the curve of disappearance of living cells is obtained.

It is evidently possible that if the constants are adjusted for each phase of growth the equation will hold good throughout the whole life period of a growth of micro-organisms. The period of retarded growth

independent of age (if this were the only factor the logarithmic law would apply), the other a factor increasing with the age, becoming twice as effective after each period of about 9½ years. Between 20—30 deaths are due about one half to the one factor and one half to the other; in later periods of life the second factor far outweighs the first.

This equation does not hold good for the earlier periods of life, when doubtless other factors are of importance.

TABLE II.

Age	Y	Z	$Z - 0{\cdot}00110$	A
20	711714	0·00199	(Z_0) 0·00089	—
21	708463			
30	673200	0·00292	0·00182	0·311
31	668682			
40	615964	0·00520	0·00410	0·332
41	608632			
50	530888	0·00849	0·00739	0·306
51	520608			
60	409518	0·0159	0·0148	0·305
61	394793			
70	246630	0·0325	0·0314	0·309
71	228844			
80	82298	0·0716	0·0705	0·317
81	69789			
90	7724	0·150	0·149	0·318
91	5470			
100	68	0·276	0·275	0·311
101	36			
			Average	0·314

especially in cases when the $X - Y$ curve is not a straight line requires investigation, and overlapping periods when for example growth and dying off take place simultaneously also deserve attention.

Summary.

Ledingham and Penfold have shown that during the lag-phase of growth of *B. coli* in a nutrient medium the time and bacilli are connected by an equation of the form $X^n = k \log Y$ where n and k are constants (the initial seeding = 1). It has been pointed out in this communication that there is a relationship between n and k, and that the above equation can be put in the form

$$X^n K^n = \log Y^n.$$

The advantage of this new equation is that K remains of the same value throughout the whole series of experiments.

In the case investigated by Ledingham and Penfold the constant of unrestricted growth is approximately equal to $2K$.

The equation can also be put in the form

$$Z = aK^n X^{n-1},$$

where Z is the "constant" of growth $\left(\frac{dY}{dX}\Big/ Y\right)$ at any time X. By suitably adjusting n and a this equation can be made to represent not only the lag-phase of growth but also the logarithmic phase, and the special phase of retarded growth when the $X - Y$ curve is rectilinear. When cell-death occurs the bacteria usually perish at such a rate that the $X - Y$ curve is logarithmic; the general equation therefore also covers this case.

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INVESTIGATIONS ON THE PREVENTION OF NUISANCES ARISING FROM FLIES AND PUTREFACTION[1].

BY F. W. FOREMAN, M.A., F.I.C.
AND G. S. GRAHAM-SMITH, M.D.

(With Plates I—V, four Text-figures and three Charts.)

CONTENTS.

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INTRODUCTION[1].

In the first part of this paper we summarize the series of preliminary experiments and observations which led us to consider that coal-tar creosote oil, alone or combined with other reagents, would prove of great use for a variety of purposes, including the prevention of putrefaction in exposed bodies, the deodorization of putrefying carcases, the destruction of fly maggots in animal refuse and manure, and the prevention of nuisances caused by flies.

In Part II we record the results of our investigations in regard to certain phenomena, such as the production of gas and odours, the exudation of fluid and chemical changes in the tissues, which precede or accompany the disintegration of the principal constituents of the

[1] These investigations were aided by a grant from the Local Government Board

body under various conditions. The importance of these phenomena and the chief factors in their causation are discussed, and the means at our disposal for estimating their effects. The mode of entry into the tissues of putrefactive organisms, and their relationship to the changes which occur in carcases, the effects of treatment of the skin and of injection of various reagents into the blood vessels are considered. Finally we summarize our views on the series of changes which occur in exposed bodies, and suggest methods by which the nuisances arising from them can be mitigated.

In Part III we consider the actions of various coal-tar oils and their constituents on maggots, and the results of treating the carcases of small and moderate sized animals exposed in the open.

In Part IV we record the results of the use of creosote oil mixtures, either in experiments devised to simulate as closely as possible conditions likely to be encountered in Public Health practice, or in warfare.

To those who are concerned with the practical application of methods for dealing with the nuisances caused by flies and putrefaction Parts III and IV of this paper may be of some value. We trust that those who are specially interested in the processes of putrefaction and the problems in connection with them will find in Part II data, which will suggest useful lines of research.

Our investigations were undertaken with the express purpose of discovering easy and practicable means for mitigating the various nuisances arising from exposed animal matter, and in pursuing this object we were compelled for lack of time to abandon, often at a most interesting stage, several series of experiments designed to throw further light on the more striking phenomena observed in decomposing carcases. Those observations and experiments which illustrate these striking phenomena, or indicate the difficulties which have to be surmounted in dealing with them, or explain the reasons, which led us to suggest the use of certain methods have been quoted at some length. Other experiments which, though of considerable scientific interest, seemed to be less intimately associated with the practical aspects of the question we have not described. The latter experiments deal with the actions of dilute acids and other reagents on bacteria, and the chemical substances in the tissues which certain species attack.

Part I. Preliminary Investigations.

The Destruction of Adult Flies.

In the early summer of 1915 we carried out a series of careful experiments in the hope of finding some method of destroying adult flies with the aid of such reagents as might be safely used in houses or by troops in the field. No satisfactory substance at once cheap, easily obtained, non-poisonous and attractive to flies was found, and we soon came to realize that even if such a substance was found its use would have little effect, except as a temporary measure, in diminishing the fly nuisance. Suitable screens together with the use of repellents would probably be more efficient than food or vapours, poisonous to flies, in keeping dwellings free from them. Since house flies seem to have a restricted range of flight and their numbers are determined by the amount of attractive material available as food for the larvae the attack should be made on the larvae, which are confined as in a trap in their breeding places, instead of allowing the adults to emerge and then attempting to destroy them. By efficient treatment of the breeding places we might hope to diminish the numbers in succeeding generations to a material extent, while the destruction of a small proportion of the adults could have little or no effect in this respect, for though fewer eggs would be deposited the competition amongst the larvae would be decreased, and the numbers emerging in the next generation would not be affected. No traps or reagents could act as efficiently as winter in destroying the adults, yet if food is available for the larvae and other conditions are favourable the swarms of adults are as numerous as ever during the succeeding year.

Owing to these considerations our attention was directed mainly to means for destroying maggots in carcases and so preventing the swarms of those species of flies whose larvae feed on such materials, but before passing to this aspect of the subject it may be of interest to quote some of our preliminary experiments with the adults.

Flies are susceptible to poisons absorbed (1) from the alimentary canal and (2) as vapours through the respiratory apparatus.

(1) *Poisons absorbed from the alimentary canal.*

The habits of flies render it impossible to observe with accuracy the effects of reagents taken by the mouth, if experiments are conducted with many individuals at a time. Some may feed well; others feed

badly or not at all; some vomit the material they have swallowed; others retain it; members of some species are more susceptible than those of others. We used various common species of flies and each experiment was conducted with a single, apparently healthy individual, so that we could ascertain the extent to which it fed, note the effects after various intervals of time, and watch the symptoms for hours without the possibility of confusion, certain to occur when several individuals are confined in one cage. Whenever any symptoms of toxic action were noticed the experiment was repeated. Our experience has shown that prolonged observations are essential since on the one hand flies which appear to be dead often recover after remaining motionless for hours, and on the other hand flies, which are apparently not affected for some time, die subsequently.

In Table I we have classified as far as possible the drugs used according to their chemical relationship.

TABLE I.

Showing the effects of various reagents on flies when taken by the mouth.

Acids.	
Sulphuric acid 1 %	Slack some hours later.
Ortho-phosphoric acid 1 % ...	No effect for 24 hours.
Meta-phosphoric acid 1 % ...	,, ,,
Carbolic acid (pure phenol) 1 % ...	Ill in 30 minutes. Recovered later.
Tannic acid	No effect in 24 hours.
Bases.	
Soda N/10	No effect in 24 hours.
Aniline saturated aqueous solution (about 3 %) + equal volume of syrup	The fly becomes ill at once. It turns on to its back, buzzes violently for a few seconds, and then lies still with occasional quivering of the legs and wings. This phase lasts about 20 minutes. If sufficient is taken death invariably follows. Probably the symptoms are due partially to effects on the respiratory system. Numerous experiments were made.
Aniline hydrochloride 2 % ...	Effects similar to those of aniline.
Monomethylaniline + 1 drop HCl	Appeared to be dead in 10 minutes; recovered in two hours.
,, ,, (watery extract) (only slightly soluble)	No effect.
Toluidine Ortho- + 1 drop HCl ...	Effects similar to those of aniline.
,, Para- + ,, ,, ...	No effect.
,, ,, (aqueous extract)	,, ,,
Zylidine (25 % in water emulsified with a trace of bile)	Dead in three minutes.

Zylidine (1 % with sugar) ...	*C. erythrocephala* no effect. *L. caesar* ill, but recovered.
Nicotine 1 % (Commercial) ...	Flies fall over on their sides while drinking and cease to move in a few seconds, except for twitchings.
" 1 % on sugar	No effect.
" 1 % sprayed on liver ...	Soon became ill, but recovered.
Hydroxylamine hydrochloride 2 %	Little, if any, effect.
Metaphenylene diamine 1 % ...	No effect.

Salts.

Strontium chloride 1 %	One fly defaecated 16 times in 45 minutes, and became very weak. Others suffered from diarrhoea, and appeared to be dead in 30 minutes.
Barium chloride 1 %	No effect within a few hours, but the flies died within 24 hours.
Barium sulphide (emulsion in water)	Died in five hours.
Copper chloride 0·2 %	No effect in 24 hours.
Manganese chloride	Flies dislike it, and it appears to possess some toxicity.
" " 1 % in syrup	Died in three hours.
Zinc chloride 1 %	Flies fed well, but soon vomited. Otherwise unaffected.
Sodium nitrite 2·5 %	Soon became ill, and died within 24 hours.
Sodium hyposulphite	No effect.
Ferric chloride 1·5 % in 5 % alcohol	Died within 24 hours.
Potassium cyanide 1 %	No effect.
Tartar emetic (strong solution) ...	No effect in 40 minutes, died in 24 hours.

Miscellaneous.

Naphthalene, sugar and water ...	Very ill in 30 minutes, but recovered.
Iodine in Potassium iodide (dilute)	No effect.
Liver of sulphur	"
Saponin 1 %	"
Formalin 2 %, Aniline 5 %, Bile 0·12 %	Very ill in a few minutes, and appeared to be dead in 15 minutes.
Aniline 5 %, Phenol 2 %, Bile 0·5 %	Very ill in a few minutes, and appeared to be dead in 15 minutes.

This table exemplifies that substances toxic to animals are not necessarily toxic to flies.

Finding aniline to possess toxic properties we persevered to some extent with experiments on adult flies with bases of the aniline family to see if toxicity could be associated in any way with chemical constitution. Orthotoluidine apparently possesses the same toxicity as aniline, but paratoluidine, in the form of its soluble hydrochloride, seems to be non-toxic. The transposition of the CH_3 group into the

para position appears to eliminate the toxicity. We had no metatoluidine at the time. The toxicity was not increased by introducing the CH_3 group into the NH_2 group of the aniline. Monomethylaniline for example was not more toxic.

(2) *Poisons absorbed as vapours through the respiratory passages.*

Many volatile organic substances appear to affect the fly through its respiratory passages, especially when they are brought into contact with the exterior of the insect. Some of these, such as ether and chloroform in moderate doses, produce temporary anaesthesia, while others produce effects which are fatal. The rapidity with which effects are produced in an enclosed space appears to depend upon the rate of evaporation. When the fluid comes into actual contact with the fly, the effect may be almost instantaneous. This is especially the case with those fluids which are capable of spreading, and entering the spiracles. We found that certain volatile organic bases of the aromatic series, such as aniline, pyridine, and such substances as crude naphtha, coal-tar oils, terpenes and volatile organic acids produced rapidly fatal effects, if brought into contact with the flies, even in minute doses.

The Destruction of Eggs and Larvae.

For reasons given in the preceding section we next turned our attention to means for destroying maggots especially in carcases.

We were so impressed with the toxic action of aniline upon flies that we decided to try its effect upon eggs, larvae and pupae, since it was cheap and easily obtainable in large quantities.

Some of our preliminary experiments, of which three are quoted, showed that very dilute emulsions of aniline rapidly killed eggs and maggots.

(1) Masses of fresh blow-fly eggs were placed on two pieces of liver and the pieces were then sprayed with 1 % nicotine solution and saturated aniline (3 %) water respectively. In the first case the maggots hatched and devoured the liver. In the second the eggs failed to hatch.

(2) 24 full grown maggots were placed in a small quantity of bran moistened with a little aniline water. All were dead, brown and soft in a few hours.

(3) No flies emerged in seven weeks from a mixed lot of fly pupae sprayed with a little aqueous solution of aniline. Controls moistened with the same quantity of water emerged in a normal manner.

After many similar experiments, which gave practically identical results, we proceeded to treat the carcases of small animals exposed in the open. It soon became evident that treatment with an emulsion of aniline, or a solution of one of its salts, would kill any maggots already present on the carcase, and prevent the hatching of any eggs that might be deposited within several days of the treatment, but to accomplish this the whole surface of the body, including the natural orifices, had to be thoroughly wetted with the solution.

After numerous experiments on the bodies of such animals as guinea-pigs, rats and rabbits we thought the efficiency of the solution might be enhanced by an increase of the strength of the aniline, and the inclusion of a substance which possessed a toxicity of a different character. To increase the proportion of the aniline over 3 % it became necessary to make up the fluid in the form of an emulsion. This we accomplished by the addition of 2 % soft soap or 0·5 % ox bile[1]. The latter substance has the advantage of causing the fluid to spread quickly and evenly over the skin and among the hair roots, and facilitates penetration. As a second toxic agent we chose carbolic acid, which differs from aniline in being acid in character and at the same time does not form a stable salt of aniline in such a solution. We hoped in this way to increase the efficiency of the solution. We compared solutions of the soluble salts of aniline, such as the hydrochloride and acetate, with 5 % emulsions of aniline and found that though these salts were toxic they did not give such good results as the free aniline. As however penetration into the skin of the carcase would be assisted by a soluble salt we arranged that our fluid should contain one part of the aniline in the form of aniline acetate and four in the free state to exercise its undoubted repellent action on flies.

Finally a fluid of the following composition, which we have termed "Solution A," was prepared:

Solution A.

Aniline	50 c.c.
Glacial acetic acid... ...	6·6 c.c.
Phenol	5 grms.
Bile	5 c.c.
Soft soap	20 grms.
Water up to	1000 c.c.

[1] We believe that ox bile might be used with considerable advantage for the preparation of insecticides and fungicides intended for application to rough surfaces.

In this solution the acetic acid is added in order to convert one-fifth of the aniline into the form of its acetate, the soft soap to produce an emulsion and the bile to assist diffusion and penetration.

Before treating carcases with this fluid we exposed them until maggots were present, and in many cases waited until the maggots were full grown, especially in the natural openings of the body. Then sufficient only of the fluid was sprinkled over the surface to completely wet the coat after roughly rubbing it in with the fingers. Finally a little of the fluid was poured into the mouth, nostrils, eyes, anus and genitalia. In some cases the carcases were opened and the thoracic and abdominal organs exposed. When this was done some of the fluid was poured into the thoracic and abdominal cavities. A guinea-pig weighing 400–500 grms. received about 100 c.c. It should be noted here that the smaller the body the greater is the ratio of surface to weight, and consequently the amount of fluid required for the treatment of a small carcase is greater per unit of weight than for a larger one. The nature of the coat makes an appreciable difference in the quantity required, the woolly coat of the rabbit absorbing much more than the short coat of the guinea-pig. The presence of bile in the fluid so facilitates the spread and penetration of the fluid that the amount necessary to thoroughly wet the coat and skin is greatly reduced, and the differences due to coats of varying character minimised.

Experiments with solutions containing aniline.

It seems unnecessary to quote in detail the very numerous experiments we carried out and our reasons for undertaking them, and therefore we have been content to summarise briefly the steps involved in the evolution of "Solution A." We feel, however, that it is desirable to describe a few experiments exemplifying our methods of observation, and the results obtained with "Solution A," and modifications of it.

The carcases were laid on the ground, in some cases exposed in the open, in others protected from the sun and rain in such a manner that the flies had free access to them. Each carcase was thoroughly examined every day, in some cases for a period of six weeks, and the results recorded. In each series of experiments an untreated carcase was included as a control, and examined daily with the others.

On 9 July an unopened carcase of a guinea-pig, not previously exposed to flies, was treated with an emulsion containing aniline 5 % and soft soap 5 %. It was examined daily till 14 August, and neither eggs nor larvae were seen on it at any time. On this date the carcase was opened and found to be moderately decomposed. When

re-examined on 3 Sept., it was found to be much decomposed, and many maggots were present under the skin. *Note.* The carcase remained free from eggs and maggots for five weeks, but when untreated surfaces were exposed eggs were deposited and maggots developed.

Similar results were obtained with a rabbit treated in the same way at the same time.

Controls were reduced to skeletons within a week.

Aniline in conjunction with other reagents.

The experiments quoted below were carried out synchronously on the carcases of guinea-pigs, which had been opened and exposed for 48 hours, and contained innumerable maggots.

I. 100 c.c. of the following solution was applied. Aniline 5 c.c., nitrobenzene 1 c.c., bile 0·25 c.c., water up to 100 c.c. By the next day all the maggots were dead. Two masses of eggs were deposited on the second day. No maggots were found until the 16th day, when a few were present in the mouth. By the 21st day the body was much decomposed.

II. 70 c.c. of the following solution was applied. Aniline 5 c.c., formalin (40 %) 2 c.c., bile 0·2 c.c., water up to 100 c.c. After 24 hours some of the maggots were still alive, although many were dead. On the 2nd day a few maggots were still alive in the mouth. On the 21st day the remains of the carcase were much decomposed, but no maggots or eggs were seen.

III. 70 c.c. of an aqueous solution of aniline acetate corresponding to 5 % aniline were applied. By the next day all the maggots were dead, and none were seen subsequently on the carcase. By the 21st day the carcase was somewhat decomposed but not to the same extent as I or II.

IV. 70 c.c. of a 4 % solution of acetic acid were applied as a control to III. The maggots were unaffected, and the carcase was completely eaten on the 5th day.

V. 100 c.c. of the following mixture was applied. Aniline 5 c.c., glacial acetic acid 0·66 c.c., bile 0·5 c.c., phenol 0·5 c.c., water up to 100 c.c. Next day all the maggots were dead. Some eggs were deposited on the 9th day. On the 21st day no maggots were seen and the carcase was much decomposed. On the 28th day many maggots were found in the coat between the skin and the ground.

VI. On the same day the carcase of another guinea-pig in the same condition was treated with 100 c.c. of "Solution A," which only differs from the above in containing 2 % soft soap. Next day all the maggots were dead. On the 10th day a batch of eggs were laid. No maggots hatched up to the 28th day and the body was hard and appeared mummified. Later some maggots were found between the skin and the ground, and some of these subsequently attacked the carcase.

We found in various experiments that 0·5–2·0 % phenol together with 0·5 % bile had little, or no, effect upon maggots feeding in a carcase. Nevertheless other experiments led us to believe that when present with aniline in a mixture the toxicity of the fluid and its antiseptic properties were increased.

Thinking that it might be desirable to ascertain the extent to which the aniline penetrated into the tissues after application to the skin in the usual manner we carried out the following experiment. An unopened carcase of a guinea-pig with maggots in the mouth and on the coat was treated with 100 c.c. of "Solution A" (without soft soap), and left on the ground for three days. The skin was then carefully removed in order to avoid contamination of the underlying tissues, and the (*a*) skin, (*b*) lungs and stomach, (*c*) abdominal organs, (*d*) the remainder of the carcase, and (*e*) the soil underlying and immediately surrounding the carcase to a depth of three inches were separately placed in flasks with water, and distilled with steam. Three successive fractions in each case were titrated with bromine water standardised against pure aniline hydrochloride. The following results were obtained:

(*a*)	Skin	0·347	grms.
(*b*)	Lungs and stomach ...	0·039	,,
(*c*)	Abdominal organs	0·082	,,
(*d*)	Remainder of carcase ...	0·420	,,
(*e*)	Soil	1·049	,,
		1·937	,,

In spite of the fact that only two-fifths of the aniline was accounted for the experiment demonstrates that some absorption into the tissues takes place. Some was probably lost by evaporation and some by diffusion into the surrounding soil, and doubtless more would have been obtained if the tissues had been ground with sand.

The experiments we have quoted show the usual results obtained by treatment with such a fluid as "Solution A" during at least three weeks, but we must emphasize that to obtain such results a thorough treatment is necessary since the maggots may gain entrance at an untreated place. Further the atmospheric conditions seem to influence the results. Hot and sunny weather tends to mummify the bodies, and rain to letch away the soluble constituents of the fluid and to hasten putrefaction. Given favourable conditions the fluid was efficient, even when diluted with twice its volume of water. Sometimes maggots present in the cavities were killed before they could reach the surface, in which case they soon turned brown. Those not instantly rendered immobile came out, but invariably died either on the carcase or in its vicinity, though in some cases death was delayed for hours.

At this stage we had achieved a considerable measure of success in the purpose, the killing of maggots in carcases, for which the

experiments had been devised, but throughout the progress of this part of the work we felt that much more could be done in mitigating the nuisances arising from flies and putrefaction if means could be devised for arresting, or at least materially diminishing, putrefactive changes, and for repelling flies from decaying carcases. Several competent observers, who closely followed our work, expressed the same opinion. From this point onwards our attention was directed to the study of putrefaction with this aim in view, and we have published the foregoing pages mainly to illustrate the stages through which our researches passed until we found in the creosote oil mixture, we have called "Solution C," a fluid which combines together with the capacity for destroying maggots other useful functions of great importance in preventing nuisances arising from exposed carcases. Nevertheless "Solution A" might be of use under exceptional circumstances when it was desired to prevent flies from breeding in carcases without appreciably checking the progress of putrefaction. Here we should like to point out that the burial of carcases containing eggs or maggots does not prevent the subsequent emergence of the flies, for the maggots continue to develop and when full fed make their way towards the surface of the ground where they pupate (Graham-Smith, 1916, p. 503). It is therefore advisable to treat all carcases with some maggot-destroying fluid before burial.

Early Attempts to check Putrefaction.

"Solution A," which contains two antiseptic agents in phenol and aniline, when applied to the skin appeared to check putrefaction to a slight extent, and we proceeded to determine whether the addition to it of larger quantities of phenol and other antiseptics would check putrefaction still further. We therefore experimented with the following variations of "Solution A," which we called "Solutions B 1 and B 2."

"Solution B 1" consists of "Solution A" with 1 % of crude carbolic acid added. This solution showed no advantage over "Solution A" in several experiments carried out with carcases of puppies, rabbits, etc.

"Solution B 2" consists of "Solution A" with from 1 to 3 % of bone oil added.

The carcases of three guinea pigs with the abdominal organs exposed and containing numerous maggots were each treated with 100 c.c. of "Solution B 2" containing 1, 2 and 3 % respectively of bone oil. In each case nearly all the maggots were killed with great rapidity. During a month's observation neither eggs nor

maggots were seen on these bodies. The bodies were protected from the rain, and on all their exposed surfaces a crust formed below which putrefaction proceeded. With such small bodies we found difficulty in comparing the odours from different carcases, but we believe that bone oil tends to diminish the putrefactive stench. We also observed in these and in several similar experiments that flies were less inclined to visit these carcases than others.

Bone oil contains various organic bases including aniline, pyridine and its homologues as well as various nitriles, hydrocarbons, etc. It therefore contains several substances which exercise repellent action on flies and are toxic to maggots.

A number of experiments with other disinfectants indicated that putrefaction could not be checked to any material extent by the application of aqueous solutions to the skin. This is hardly surprising since rain water or water arising by capillarity from the ground dilutes the fluid and letches away both the disinfectant and those products of bacterial activity which tend to check putrefaction. As we will show later the presence of water and a favourable temperature are the most potent factors in accelerating putrefactive processes. We entertain little doubt that on the one hand any agent which softens the skin aids the maggots in gaining entrance into the body, and on the other hand any agent which hardens the skin tends to prevent their entrance and retards the liquefaction of the superficial tissues, a change which seems to be correlated with the admission of water and air.

Internal combined with external treatment.

Seeing that surface applications with aqueous solutions were of little value in retarding putrefaction, we injected disinfectants both into the serous cavities and into the blood vessels in the hope of evenly distributing the antiseptics throughout the tissues and thus checking the action of putrefactive bacteria. One series of experiments may be quoted:

Four rabbits, treated 24 hours after death, were exposed in the open. The weather became wet and unfavourable.

I. Weight 7 lbs. 4 c.c. crude carbolic acid containing aniline 2 % and bile 1 % were injected into the thoracic cavity and 4 c.c. into the abdominal cavity. 3 c.c. of "Solution B 1" were injected subcutaneously at different situations, and the surface was treated as usual with 300 c.c. of "Solution B 1." By the 5th day the body was becoming distended with gas. By the 8th the hair was coming off everywhere, and numerous eggs were present on the back. On the 9th day thousands of small maggots were present under the fur. On the 12th day the body was much decomposed, and on the 21st day horribly putrid.

II. Weight 5 lbs. The abdominal cavity was opened. Proportionately to its weight this carcase was treated in the same manner as I. On the 8th day the abdominal wound was green, the hair was coming off and there was some odour. On the 9th day thousands of small maggots were present under the hair of the back. On the 21st day putrefaction was less advanced than in I, but a horrible stench arose when the body was disturbed.

III. Weight 5 lbs. The blood vessels were injected through the carotid artery with 3 c.c. of crude carbolic acid containing aniline 2 %, and bile 1 %. The surface treatment was the same as for II. On the 8th day the skin was green. On the 12th day the under surface was very putrid, and there were many small maggots present. By the 21st day the maggots were very numerous.

IV. Weight 5 lbs. The blood vessels were injected through the carotid artery with 50 c.c. of 5 % formalin, and the surface treated in the same manner as I. On the 7th day a few maggots were present. By the 12th day numerous maggots of all sizes were working actively. On the 16th day the body was much decomposed. On the 21st day it was so putrid that the legs came off at the slightest touch and the stench was horrible.

These experiments illustrate the effects of rain in decreasing the efficiency of aqueous solutions applied to the surface in checking the development of maggots.

We concluded from many such experiments that satisfactory results could not be obtained from injections of disinfectants, if means were not taken to cut off the access of water to the carcase from all sources, rain, dew, humid atmosphere, soil saturated with water, and water rising by capillarity from an unsaturated soil. In seeking the best way of achieving this purpose we conceived the idea of applying over the whole surface of the carcase a film of some insoluble oily substance. As a natural consequence of this decision it occurred to us that the oil should be made a vehicle for applying agents dissolved in it possessing fly deterrent, maggot destroying, deodorising and antiseptic properties. Now substances identical with, or similar in nature to, those we have already shown to possess some of these properties are present in coal-tar distillates, which constitute the cheapest substances obtainable possessing the desired oily character. We were influenced in our selection by the following important considerations: the fluid should have (*a*) a high percentage of disinfectants, (*b*) a low rate of evaporation in order to ensure that the film should remain operative as long as possible, (*c*) a high flash point so as to avoid danger from fire, (*d*) no unpleasant smell, and (*e*) no undesirable poisonous properties. After some preliminary experiments with several coal-tar products we concluded that creosote oils would be the most suitable for our purpose.

Creosote oils from different sources show very considerable variations in composition, depending upon the kind of tar, the method of preparation, and the extent to which they are contaminated with other products. In the best managed works the creosote oil is uniform in character, while "in some works every residue which cannot be used for any other purpose finds its way into the creosote oil well."

The following varieties of creosote oil can be distinguished:

Coal tar creosote oil "London make"...	4— 7 %	tar acids
,, ,, "Country make"	14—18 %	,,
Blast furnace tar creosote oil	20—35 %	,,
Water gas tar creosote oil	practically no tar acids.	

Though the blast furnace tar creosote oil contains the highest percentage of tar acids it appears to be unsuitable in other respects, being a thin liquid lighter than water which evaporates faster than the coal-tar creosote oils, and lacks oilyness. We chose therefore a trustworthy country make which we found on analysis to contain tar acids 13·85 %, bases 3·94 %, the remainder consisting of oily hydrocarbons, traces of water, etc.

Treatment with creosote oil has enabled us not only to suggest methods of practical value, but also to study putrefactive processes in a manner previously impossible. During the progress of our earlier experiments we felt the need of satisfactory standards when attempting to compare the results obtained by various methods. Written descriptions of the appearances noted are necessarily vague and lacking in precision and the element of personal bias, which cannot be eliminated, leads to the introduction of considerable errors. Moreover the manifestations of putrefaction vary both in degree and kind in carcases kept under similar conditions, and to a far greater extent in carcases kept under different conditions. We therefore decided to devote some attention to the study of the more important phenomena which accompany putrefaction. These researches were carried on partly concurrently with attempts to find practical methods for dealing with decomposing carcases, and partly after these methods had been devised and tested in the field.

For descriptive purposes we felt it would be best to separate the more scientific from the more practical aspects of the work, and we decided to deal first with the former in Part II. By this arrangement we hope the procedures adopted and the conclusions arrived at in Parts III and IV will be rendered more easily intelligible.

Those, however, who desire to follow the more practical part of the work in the order in which it was carried out may prefer to pass immediately to Parts III and IV in which it is described.

Conclusions.

1. Attacks on the adult fly are not likely to produce appreciable effects on the numbers in succeeding generations.

2. In order to diminish the fly nuisance the eggs and larvae should be destroyed in the breeding places, where they are confined as in traps.

3. Watery emulsions or solutions of larvicides, if properly employed, kill eggs and larvae in carcases, but soon lose their efficiency in the presence of water.

4. Larvicides of an oily nature retain their potency for long periods and are, therefore, the most suitable agents to employ.

Part II. Investigations on Putrefaction.

In order to obtain some knowledge of the early stages of putrefaction and the factors which influence them we made careful observations on the bodies of small animals. Some of these were kept indoors in a dry atmosphere at temperatures ranging between 60 and 80° F., others in a moist atmosphere at 26·5° C., others were placed outside on the ground covered to protect them from sun and rain, others on the ground without protection, and others exposed and frequently wetted. Daily observations were made and notes taken, and some of the carcases were dissected at different stages. All were dissected when it was thought that the observations had been carried on sufficiently long. Most of these experiments were carried out in the summer and early autumn.

External Manifestations of Putrefaction.

The first signs of putrefaction usually noticed in the carcases of small animals are distension of the abdomen and protrusion of the anus. The body soon assumes a cylindrical shape owing to the extension of the gas throughout the subcutaneous tissues. The period during which distension remains at its maximum depends upon external conditions, principally temperature and moisture.

During the period of distension a clear yellowish fluid exudes and raises the hair and superficial epidermis from the deeper layers of the skin over an area on the left side, approximately corresponding to the cardiac end of the stomach. On removing the hair and superficial epidermis over this area the follicles appear as pits in the smooth, moist, deeper layer of the skin. From the patch described the process usually extends over the abdomen and later over the whole area of the skin, resulting in the loosening of the hair everywhere. The distension gradually subsides and concurrently large quantities of reddish fluid escape from the body, and the odours of putrefaction become more evident. The source and nature of these odours will be discussed later (p. 156). After this the skin desquamates and the soft tissues of the carcase gradually liquefy, if sufficient moisture is present.

In the early stages of distension the skin especially over the abdominal area exhibits a bluish green discoloration. It has been suggested that this discoloration is due to the action of sulphuretted hydrogen, one of the earliest gases to be evolved, upon the iron-containing pigments in the blood. The sulphuretted hydrogen probably arises from free cystine. We may point out that many organisms produce green colonies on blood agar plates when carbohydrates are present. Ruediger (1906) suggests that this is caused by the action of organic acids produced from the carbohydrates on the red corpuscles or haemoglobin. The discoloration rapidly extends over the whole surface of the carcase, and throughout this stage numerous organisms are found in the green tissues.

The influence of maggots on carcases.

Fly maggots, besides eating up the carcases, may in the meantime exert great influence in other ways. Young maggots seem to be able to live on the surface of the skin upon the excluded fluid, and may reach a large size apparently without any other food. When maggots gain entrance into the carcase they assist in the distribution of the bacteria and ferments in the muscular tissues. If, as we believe, the skin is more important than the alimentary canal as a source of putrefactive organisms this action of the maggots would tend to hasten putrefaction in the muscles. On the other hand the opening up of the carcase tends to introduce aerobic conditions. We have made no attempt to investigate the extent to which the presence of maggots influences putrefaction.

Internal Appearances.

The stomach and the liver are the first organs to show decided changes. A red-purple patch of discoloration soon appears over the cardiac end of the stomach, and this area becomes so soft that the organ ruptures upon the slightest manipulation. Eventually in many cases this area of the stomach wall becomes completely dissolved. A corresponding patch appears on the parietal peritoneum and the colour extends through the abdominal wall. The fluid which exudes from the body first escapes from this area.

The liver soon becomes soft, and at an early stage the formation of gas results in a honeycomb condition, which is sometimes confined to the superficial lobes. At this stage the organ floats in water, although much of the gas is discharged into the peritoneal cavity. Finally the gas escapes leaving the liver thin and flat. The spleen and kidneys become soft, but their substance exhibits no evidence of gas formation. The intestinal walls seldom rupture, until a late stage in decomposition. Changes occur slowly in the thoracic organs. The muscles exhibit their characteristic colour and consistency long after visible changes have occurred in the other organs. The muscles retain the red-pink colour if anaerobic conditions prevail, but become gray when exposed to the air, possibly through oxidative changes. They also lose their firm consistency, first becoming soft and easily detached from the bones and later diffluent. The change in consistency becomes evident earlier in the softer muscles, such as the psoas.

When a carcase which has reached the point of maximum distension with gas is opened large quantities escape from the peritoneal cavity. Gas is usually found in the intestines, the degree of distension probably depending upon the kind and amount of food eaten some hours previous to death. Separate large pockets of gas occur in the retroperitoneal tissues, and gas is found between the layers of muscle and in the subcutaneous tissues, producing subcutaneous emphysema.

In most carcases extensive oedema of the subcutaneous and other tissues occurs. The conditions found on the dissection under water of the body of a guinea pig which had reached this stage are given below.

When a perforation was made in the skin of the shoulder about 10 c.c. of gas escaped. The gas was evidently in the interstices of the subcutaneous tissue, since more could be forced out by pressure. About 40–50 c.c. came out of the peritoneum, and the whole intestine was considerably distended with gas. Its mucous membrane seemed to be destroyed in most places. Much gas was present in the pleural cavities.

After opening the peritoneal cavity gas was still present in the subcutaneous and retroperitoneal tissues. It was also present in the intermuscular connective tissue of the limbs. No gas was found in the kidney which sank in water. The liver was soft and less bulky than normal, and showed large numbers of small gas vesicles. It floated in water. After the intestines and stomach had been removed and all the larger and smaller cavities containing gas opened the carcase still floated in water.

Gas Production in the Carcases of Small Animals.

Method of estimation.

The animals were killed and the carcases, supported on glass pedestals about two inches in height, so as to keep the bodies above any fluids which might drain from them, were placed head downwards in wide mouthed bottles (*A*) fitted with air-tight rubber bungs, pierced for delivery tubes. The bottles were arranged in a constant-level water bath (*B*) kept at a uniform temperature of 26·5° C. by means of a regulator. A delivery tube (*C*) connected each of these bottles with another bottle (*D*) of 800 c.c. capacity acting as a receiver. The receiver was fitted with a rubber cork with three perforations. The delivery tube (*C*), which was cut off level with the bottom of the cork, passed through one. Through another a tube (*E*) passed and was prolonged for one inch below the bottom of the cork. The exposed end of this tube was fitted with a glass tap, and beyond this with a piece of rubber tubing (*F*), by means of which it could be attached to a gas measuring burette. The extension of the tube below the cork was for the purpose of leaving a gas cushion, so as to ensure against water being sucked backwards through the delivery tube into the bottle (*A*), when the atmospheric pressure rose. The third opening admitted a siphon (*G*) passing from the bottom of the receiver and connected to an aspirator (*H*). Twelve such systems were connected with the same aspirator, thus reducing as far as possible the absorption of gases by the water, and rendering the conditions comparable. After all the connections had been made and sufficient time had elapsed for a temperature equilibrium to be established the glass tap was opened, and the aspirator raised so as to permit the water in each receiver to rise to the lower ends of the tubes (*E*). The taps were then closed and the aspirator lowered until the level of the water in it was three inches lower than in the receivers. The tube (*E*) was now connected with the measuring burette and the level of the water in each receiver exactly adjusted. The systems were finally tested during some hours for leakage by observing whether

the water kept at the same level in the receivers. It will be observed that there was a slight negative pressure in the receivers until they became half full of gas.

Every day the measuring burette filled with water was connected with the tube (*E*) the bulb lowered and the gas withdrawn until the water had risen to its original level, and the collected gas measured. Owing to the great volumes of gas produced during the 3rd, 4th and 5th

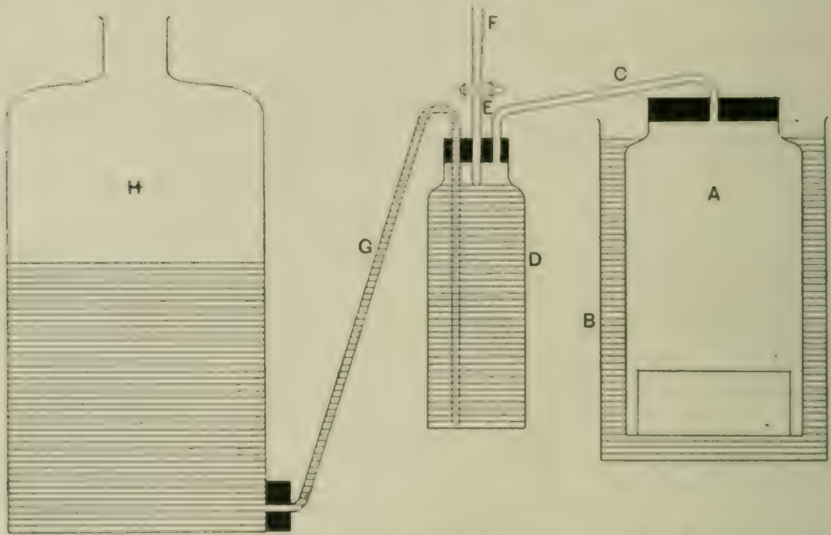

Fig. 1. Diagram of apparatus for collecting and measuring the gas evolved from small carcases.

A. Bottle containing the carcase. *B*. Constant-level water bath. *C*. Delivery tube passing to the receiver *D*. *E*. Tube through which the gas passes into the measuring burette. *F*. Rubber tube for attaching the gas measuring burette. *G*. Siphon passing to the aspirator *H*.

Twelve such systems were attached to the aspirator, but only one is shown in the diagram.

days two measurements had to be made on these days. The volumes measured were not corrected for temperature and pressure as the systems were under the same conditions and the experiments were all comparable. Each series of observations were continued for three weeks.

At first the conditions in the bottle (*A*) were aerobic, but became anaerobic when all the air had been expelled. The atmosphere was probably saturated with water vapour.

We fully realise that the conditions in these bottles differed widely from those prevailing outside.

When a small carcase is exposed outside in summer time its temperature is higher by day than by night. In the day time the bacteria which prefer a high temperature tend to increase, and during the night those which prefer lower temperatures. Profound changes result in relation to such conditions as the proportions of various species of bacteria present at different times and their products, symbiotic relationships, the consequences of bacterial antagonism, etc., and affect the order, rate and degree of putrefactive processes. The drying of the surface in warm weather, the action of sunlight, the aerobic conditions prevailing at any rate externally and absorption by the ground of exuded fluids containing products of bacterial action, which if allowed to accumulate would check bacterial growth, exert influences difficult to estimate. In large carcases these factors probably play a less important part.

While the method we have described fails to simulate natural conditions with precision it affords a very stringent test of the action of antiseptics, since all the circumstances are very favourable for rapid putrefaction.

Results of experiments for estimating the gas produced in small carcases.

Primarily our experiments were designed to show the rate of gas formation in small carcases, untreated or treated in various ways, and in some of the more important organs. We carried out three series of experiments and quote the results of two in detail.

In series I, quoted in Table II, an intact carcase (A) was compared with one in which the abdominal cavity had been opened (B), with one from which the blood had been drained by opening the large vessels of the neck (C), with one from which the stomach and intestines had been removed (D), with one from which the liver, stomach and intestines had been removed (E) and one from which these organs and the skin had been removed (F). When removing the organs the greatest care was taken to avoid gross contamination with their contents by employing double ligatures and dividing the tissues between them with a cautery. Also in the case of the body from which the skin was removed precautions were taken to avoid undue contamination by removing as much as possible of the hair by plucking, and singeing off the remainder before any incision was made. The liver, stomach and intestines (G) removed with as little disturbance as possible were placed in a bottle, a stomach and intestines (H) in another, and a stomach and intestines (I) opened in several places in a third.

TABLE II.

Showing the volumes of gas produced daily from the carcases of small animals. (Series I.)

Days	Atmospheric pressure in mm.	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
1	733	52·3	39·3	136·1	41·6	35·8	16·6	39·4	41·6	106·1
2	736	47·7	6·7	413·1	539·6	48·0	228·4	121·0	49·1	104·9
3	747	63·4	537·5	477·5	811·9	405·1	531·8	97·0	66·9	53·9
4	746	172·7	511·4	421·9	375·6	212·8	238·9	26·9	66·5	23·7
6	755	458·5	186·0	357·0	176·8	249·9	216·5	13·2	54·5	0
7	758	451·8	115·0	471·5	123·4	144·4	114·0	19·1	33·6	0
8	752	220·9	75·1	120·3	60·7	69·1	60·7	92·8	26·2	0
9	747	238·1	95·4	163·7	113·0	80·1	72·0	49·4	30·0	0
10	763	105·1	27·7	25·0	45·7	20·1	18·8	16·5	7·5	0
11	766	238·7	47·0	78·4	67·2	45·2	30·3	32·5	14·9	0
12	764	210·1	48·3	42·5	47·6	36·9	33·2	34·0	9·1	0
13	769	197·3	31·1	22·3	33·5	19·4	19·2	25·7	0	0
14	769	142·6	49·8	41·6	46·5	40·2	24·3	46·5	9·2	0
15	755	142·0	47·6	46·9	38·7	36·9	33·1	55·7	8·2	0
16	766	41·5	20·2	0	0	0	0	37·2	0	0
17	774	40·0	25·3	1·9	9·4	5·6	0	38·1	0	0
18	773	68·6	49·6	15·8	19·1	20·8	9·9	25·3	0	0
19	767	65·4	58·0	32·9	33·1	26·6	35·4	20·5	0	0
20	769	33·1	31·4	11·2	10·1	9·4	8·2	3·6	0	0
21	758	69·2	65·8	32·7	32·3	27·2	25·6	14·7	0	0
22	770	12·2	22·7	0	0	0	0	0	0	0
Total gas produced		3071·2	2090·9	3112·3	2625·8	1533·5	1716·9	809·2	417·3	288·6
Weight of body in grms.		348	331	311	252	321	172	98	100	107
Gas per grm.		8·82	6·32	10·02	7·91	4·78	9·98	8·26	4·17	2·69

Another series (II) of experiments were carried out in order to ascertain what differences in gas production, if any, would be brought about by treatment with antiseptics. An intact carcase (*J*), one in which the abdominal cavity had been opened (*K*), one from which the blood had been allowed to drain (*L*) and one from which the skin, liver, stomach and intestines had been removed (*M*) acted as controls. With these were compared a carcase treated externally with 10 c.c. of creosote oil (*N*) and one similarly treated with an equivalent quantity of 5 % aqueous emulsion of cresols in 1 % soft soap (*O*), a carcase injected through the carotid artery with 5·75 c.c. of creosote oil (*P*), and one injected with an equivalent volume of the cresols' emulsion (*Q*) and carcases with the abdominal cavity opened and the surfaces of the abdominal organs and skin treated. The skin of one (*R*) was treated with 12·6 c.c. of creosote oil and the peritoneal surfaces with 5·2 c.c.,

and the other (S) was treated with equivalent volumes of the cresols' emulsion. A liver (T) alone was placed in a bottle. The results of these experiments are given in Table III.

TABLE III.

Showing the volumes of gas produced daily from the bodies of small animals. (Series II.)

Days	Atmospheric pressure in mm.	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*
1	750	56·0	66·0	54·2	15·4	67·3	82·6	107·5	92·7	75·3	66·8	2·2
2	758	410·4	92·9	207·6	259·1	139·6	88·5	101·8	102·5	65·5	58·9	60·9
3	767	382·9	235·7	862·7	388·3	503·0	202·2	200·7	437·4	227·7	85·2	92·3
4	766	169·8	687·4	493·1	190·8	303·4	422·0	657·2	569·7	398·8	367·0	27·9
5	765	109·4	394·0	310·2	91·9	157·0	267·6	366·9	282·1	168·0	242·0	2·2
6	765	109·1	245·9	231·7	57·7	102·0	107·2	252·5	170·3	100·5	197·9	0
7	759	105·5	179·3	173·8	43·3	74·3	80·0	177·3	149·5	81·7	204·1	0
8	755	84·8	96·3	102·2	32·3	54·0	96·2	161·5	116·8	71·6	161·8	0
9	750	73·0	94·7	101·5	34·1	57·8	78·5	166·5	142·6	75·4	136·2	0
10	746	55·2	93·6	92·1	36·5	42·9	52·8	130·7	91·4	69·1	109·1	0
11	751	39·4	47·9	58·4	27·6	32·2	31·6	92·9	60·4	54·5	67·9	0
12	746	44·9	69·8	72·5	33·2	42·2	49·5	158·7	99·9	83·7	86·5	0
13	745	37·4	56·7	63·5	22·8	32·9	32·5	120·0	70·5	57·1	60·8	0
14	744	37·0	46·7	44·6	20·2	23·5	23·9	76·4	60·0	43·4	56·1	0
15	746	32·2	46·9	44·0	19·1	24·0	24·6	100·4	64·8	50·0	55·5	0
16	757	18·2	34·4	29·2	4·0	4·3	6·5	50·9	30·3	18·9	31·2	0
17	760	13·6	13·1	16·1	0	−5·1	−3·1	29·7	17·2	6·5	21·5	0
18	757	21·1	19·3	28·2	18·6	24·1	33·7	61·8	46·7	35·7	44·2	0
19	757	20·1	15·6	32·1	9·5	9·4	16·6	58·6	49·9	25·6	37·4	0
20	753	20·6	15·1	31·5	4·0	8·0	9·0	51·6	43·4	19·3	35·8	0
21	761	16·9	12·4	40·2	5·8	8·2	0	48·5	37·2	16·7	34·0	0
Total gas produced		1857·5	2563·7	3089·4	1314·2	1710·1	1705·5	3172·1	2735·3	1745·0	2160·1	185·5
Weight of body in grms		272	305	295	147	284	202	382	316	387	380	17
Gas per grm.		6·83	8·4	10·47	8·93	6·02	8·44	8·30	8·66	4·51	5·69	10·91

It will be seen that gas was produced under all the circumstances investigated and from all the organs. Great quantities were evolved during the first week, and in most cases considerable quantities continued to be evolved during the next two weeks.

Further analysis of the figures given in Tables II and III revealed some interesting facts, the importance of which can be estimated only in conjunction with the results of the examination of the carcases at end of three weeks. We proceed therefore to describe the conditions found on examining the bodies used in the second series of experiments.

Dissections of bodies used in the second gas experiment.

The carcases were removed from the bottles and examined according to a definite procedure. The following notes summarize the results of these examinations. In each case some of the fluid, which had drained from the body, and a portion of the thigh muscle were reserved for chemical analysis.

J. Intact carcase. Original weight 272 grms. 86 c.c. of red fluid had exuded. *Hair* loose everywhere. *Skin* gray and pliable. *Lungs* shrivelled; float in water. *Liver* hardly recognizable, lying like a piece of crumpled wash leather on the stomach; floats in water; sections show it to consist of a honeycomb of connective tissue. *Intestines* dark greenish-brown; rupture very easily; impossible to separate the coils. *Muscles* very soft, and pink in colour. *Smell* unbearable. *Remarks.* The carcase was supported with the head *upwards* on stones in such a manner that none of the fluid which exuded remained in contact with it. In consequence the carcase was much better preserved than other untreated bodies in the series as they were not altogether removed from the influence of the fluids by the glass supports on which they rested.

K. Abdominal cavity opened. Weight 305 grms. At least 50 c.c. of clear red fluid had exuded. *Hair* very loose everywhere. *Remarks.* The body had collapsed and part of it was immersed in the fluid. Those portions of the carcase which were immersed were semi-solid and unrecognizable, while in the remainder the muscles were just recognizable. The general condition of the carcase was such that it fell to pieces. *Smell* intolerable, with a suggestion of acetamide.

L. Bled. Weight 295 grms. At least 30 c.c. of fluid had exuded. *Hair* very loose everywhere. *Skin* very putrid. *Lungs* and *heart* completely disintegrated. *Liver* flat as in *J.* *Stomach* and *large intestine* unrecognizable. *Small intestine* recognizable and not easily torn. *Muscles* disintegrated. *Smell* intolerable, with a suggestion of acetamide. *Remarks.* This carcase was in a very advanced stage of decomposition.

M. Skin, liver, stomach and intestines removed. Original weight of body 265 grms. Portions removed weighed 118 grms. Remains placed in bottle weighed 147 grms. At least 20 c.c. of fluid had exuded. *Remarks.* The carcase was in such a condition that it could be stirred easily with a glass rod, only the bones, fibrous tissue and muscle aponeuroses being recognizable. *Smell* intolerable.

N. Skin treated with creosote oil. Weight 284 grms. 53 c.c. of red, almost odourless fluid had exuded. *Hair* slightly loose over the back and abdomen, but elsewhere nearly as firmly attached as in life. *Heart* and *lungs* soft. *Liver* moderately soft, flat and honeycombed. *Kidney* soft. *Intestines* shrunken, tough and contain no gas. *Muscles* resemble fresh muscle in appearance, colour and consistency. Some small gas pockets in the retroperitoneal tissues. *Remarks.* There was very little appearance of decomposition in this carcase.

O. Skin treated with cresols' emulsion. Weight 202 grms. 45 c.c. of brown fluid had exuded. *Hair* very loose everywhere. *Skin* soft, moist and greenish

gray. *Heart* and *lungs* soft but recognizable. *Liver* very soft, flat and honeycombed. *Kidney* soft but recognizable. *Intestines* full of gas bubbles, but retain their shape. *Muscles* very soft and putrid looking. Pockets of gas in retroperitoneal tissues. *Smell* intolerable. *Remarks.* The carcase had a very putrid appearance.

P. Injected with creosote oil. Weight 382 grms. *Smell* intolerable. *Remarks.* The carcase was in such a condition that it could be stirred with a glass rod. Except *Q* the worst specimen in the series.

Q. Injected with cresols' emulsion. Weight 316 grms. About 46 c.c. of fluid had exuded. *Remarks.* The body was in the same condition as the last.

R. Skin and peritoneal surfaces treated with creosote oil. Weight 387 grms. 60 c.c. of fluid had exuded. *Hair* firmly attached everywhere. *Skin* tough and leathery. *Heart* and *lungs* shrunken and tough. *Liver* flat, and floats in water. *Kidney* shrunken but not soft. *Intestines* shrunken and tough. *Muscles* pale pink and resembling fresh muscle in shape, size and consistency. *Smell* none, except that of reagent. Some gas pockets in the retroperitoneal tissue. *Remarks.* This carcase was extraordinarily well preserved.

S. Skin and peritoneal surfaces treated with cresols' emulsion. 45 c.c. of fluid had exuded. *Hair* loose everywhere. *Skin* very soft and detached from the underlying muscles by gas. *Heart* and *lungs* soft but recognizable. *Liver* very soft, flat and honeycombed. *Kidney* soft, but recognizable. *Intestines* contain gas bubbles and are moderately well preserved. *Muscles* very soft and strip easily from the bones. *Smell* horrible. *Remarks.* This carcase is a little better preserved than *J.*

According to these examinations the carcases may be classified into well preserved and putrid.

TABLE IV.

			Gas per grm.
Well preserved	*R.*	Skin and peritoneal surfaces treated with creosote oil...	4·51 c.c.
	N.	Skin treated with creosote oil	6·02 ,,
Putrid	*S.*	Skin and peritoneal surfaces treated with 5 % cresols ...	5·69 ,,
	J.	Intact carcase	7·82 ,,
Very putrid	*K.*	Abdominal cavity opened	7·36 ,,
	L.	Bled	10·24 ,,
	M.	Skin and abdominal organs removed	9·45 ,,
	O.	Skin treated with 5 % cresols	8·44 ,,
	P.	Injected with creosote oil	8·3 ,,
	Q.	Injected with 5 % cresols	8·66 ,,

Results of experiments on gas production. Untreated carcases.

In Table V we have given the total quantity of gas produced per unit weight in three weeks in three series of experiments, and the proportion produced during the first week.

TABLE V.

Showing the total quantity of gas produced by organs and treated and untreated bodies per unit weight in three weeks, and the proportion produced in the first week.

	c.c. of gas per grm				Percentage of the total gas given off during the first 7 days			
	Series I	II	III*	Mean	I	II	III*	Mean
Organs.								
Liver	—	11·28	13·0	12·14	—	100	100	100
Liver, stomach + intestines	8·26	—	8·04	8·15	50·6	—	100	75·3
Intestines opened... ...	2·75	—	6·55	4·65	98·2	—	70·8	84·5
Stomach + intestines ...	4·17	—	4·3	4·23	81·1	—	66·3	73·7
Untreated carcases.								
Bled	10·02	10·47	—	10·24	83·4	75·5	—	79·4
Stomach + intestines removed	10·42	—	—	10·42	81·1	—	—	81·1
Skin and organs removed	9·98	8·93	—	9·45	91·9	79·5	—	85·7
Intact	8·82	6·83	—	7·82	47·8	72·5	—	60·1
Abdomen opened	6·32	8·4	—	7·36	70·4	74·2	—	72·3
Liver, stomach, intestines removed...	6·12	6·88	—	6·5	80·5	—	75·9	78·2
Treated carcases.								
Injected "5 % cresols" ...	—	8·66	—	—	—	65·9	—	—
,, creosote oil ...	—	8·3	—	—	—	58·8	—	—
Skin "5 % cresols" ...	—	8·44	—	—	—	73·3	—	—
,, creosote oil	—	6·02	—	—	—	78·7	—	—
,, + peritoneum "5 % cresols"	—	5·69	—	—	—	56·6	—	—
,, ,, creosote oil	—	4·51	—	—	—	64·0	—	—

* The experiments of series III have not been quoted elsewhere.

It will be noticed that in nearly all instances in which two experiments were carried out with the same materials the results were similar. The exceptions include the experiments with the intestines opened in regard to the total quantity of gas produced, and with the liver, stomach and intestines in regard to the rate of production. Differences in the nature and amount of food consumed before death would be sufficient to account for these discrepancies. The other notable exception is the difference in the rates of production exhibited by the intact carcases. The conditions in these two experiments, though apparently similar, differed fundamentally. In the experiment in series I the carcase had dropped to the bottom of the bottle, and was immersed in the fluid which exuded from it, while in the experiment of series II it was supported clear of the fluid.

Considering first the abdominal organs it will be seen that the liver shows the highest gas production (12·14 c.c.) per unit weight, and that

the whole of the gas is produced during the first five days of the experiment. The stomach and intestines yielded much smaller quantities (4·24 c.c.). The liver, stomach and intestines, removed with as little disturbance as possible, gave twice as much gas (8·15 c.c.) as the stomach and intestines alone. The gas produced from the liver in this experiment would only account for about one-quarter of the increase. From this experiment and from others to be given later we are of opinion that the apposition of these organs results in direct communication of organisms and ferments, which conduces to greater gas formation.

If we turn to the untreated carcases all became putrid and yielded large quantities of gas per unit weight, the largest being given by those from which the most blood escaped during the manipulations. Possibly this result was due to the removal of a part of the bactericidal substances, which are present in the blood. The removal of the liver together with the intestines resulted in a decrease in the evolution of gas.

Origin of the gas.

Before considering the results of treatment we may with advantage discuss the origin of the gas, which is produced at various times. In the liver relatively large quantities of carbohydrates are accumulated. Apart from the liver, the blood and tissues as well as the contents of the alimentary canal contain carbohydrates. Glycogen, which is present as a reserve food material in the organs, especially in the liver and muscles, is rapidly hydrolysed to dextrose in the body after death. The liver of such an animal as the guinea-pig becomes glycogen free in a very short time. Many species of intestinal organisms produce gas very rapidly from carbohydrates in cultures, and as we will show later such organisms are in a position to attack the carbohydrates found in the body.

Carbohydrate material would also become available from the gluco-proteins, the prosthetic group of which is easily split off by hydrolysis, and from the nucleic acids, depending upon the rate of action of the autolytic ferments.

The figures given in Tables II and III indicate that the daily production of gas is largest on the 2nd, 3rd, 4th and 5th days, when control observations show only slight visible evidence of muscle degeneration. We therefore consider that in the early stages the gas arises mainly as the result of the action of organisms on the carbohydrate material. The fact that the liver yields the whole of its gas within four days supports this view. The early production of sulphuretted hydrogen

indicates however that other constituents of the body, such as cystine, may be attacked to some extent in the first few days.

We consider that the gas evolved during the later periods is due chiefly to the action of organisms upon the degeneration products of the principal nitrogenous constituents.

An early high yield of gas may bear little, or no, relation to putrefaction as evidenced by dissection and chemical analysis (p. 152) at the end of three weeks, but a total high yield indicates considerable changes.

The results of experiments on gas production. Treated carcases.

Now turning to the treated carcases we find that all those treated with the cresols' emulsion were much decomposed at the end of three weeks, and, with the exception of one in which the peritoneal surfaces were treated, yielded large total volumes of gas.

The application of the antiseptic to the peritoneal surfaces may affect gas production in two ways, by interrupting the direct passage of organisms through the intestinal walls to the surrounding organs and by destroying organisms subjected to its influence, or possibly by partly inhibiting their zymogenic functions.

Injection into the blood vessels of small quantities of 5 % cresols' emulsion or of creosote oil gave poor results perhaps partly owing to the accumulation of these fluids in the larger vessels and partly to the fixing of the phenolic constituents by albuminous substances. Larger injections might have given better results (p. 202). On the other hand treatment of the skin with creosote oil and especially application to the peritoneal surfaces combined with skin treatment gave excellent results with small total yields of gas, 6·02 c.c. and 4·51 c.c. per unit weight respectively. The former procedure, while not interfering with the carbohydrate fermenting intestinal organisms, prevents the invasion of putrefactive organisms from the skin. We find, as might be expected, a high rate of early gas formation. Subsequent examination reveals the intestines to be tough, indicating that little putrefaction has occurred, and dry owing to the draining away of the fluids. The treatment of the peritoneal surfaces with creosote oil acts more efficiently than treatment with 5 % cresols' emulsion, and, combined with the checking of the invasion from the skin, gives astonishingly good results.

These experiments together with those quoted in Part III lead us to think that the putrefactive bacteria invade the tissues mainly from the exterior, and that the bacteria responsible for early carbohydrate

fermentation are mainly intestinal in origin. The sodden area of skin through which the fluid first exudes probably constitutes the chief early portal of entry of the organisms present in the skin.

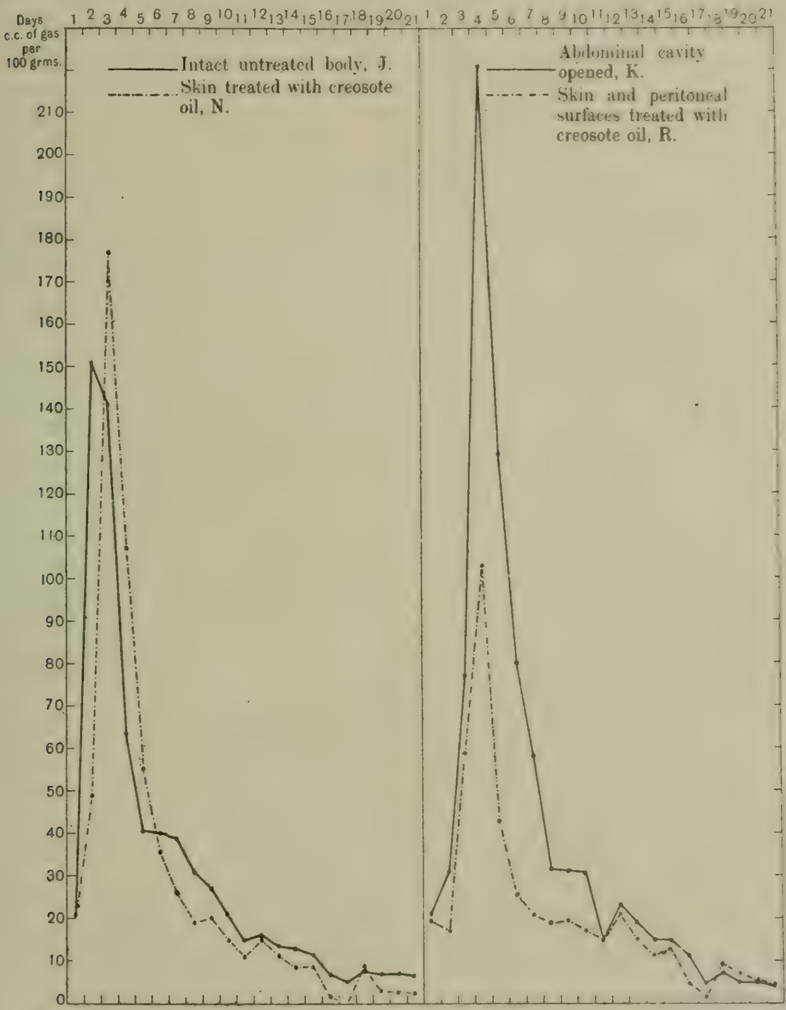

Chart 1. Showing daily production of gas per 100 grms. in the intact carcase (J) compared with the carcase treated externally with creosote oil (N), and in the carcase with the abdominal cavity opened (K) compared with the carcase with the skin and peritoneal surfaces treated with creosote oil (R).

In Chart I it will be seen that the effect of the skin treatment with creosote oil upon the initial voluminous gas formation is negligible,

but the skin-treated body gave smaller daily yields of gas in the later stages. On the other hand treatment of the skin and peritoneal surfaces with creosote oil resulted in a great decrease in the initial gas production, which confirms our view that the organisms responsible for this early gas production emanate from the intestines. Again the treated body gave smaller daily yields of gas in the later stages.

The Distribution of Intestinal Bacteria in the Organs during Life and after Death.

Our views in regard to the distribution of intestinal organisms in the tissues are based on certain carefully conducted experiments in which the opportunities for accidental contamination were reduced as far as possible. The method adopted for removing and crushing portions of organs, under sterile conditions, were those devised and used by Cobbett and Graham-Smith (1910, p. 6) in their investigation of Grouse Disease. They found that bacteria of intestinal origin were rarely present in the livers and other organs of grouse unless the coeca have been injured by the presence of large numbers of worms, *T. pergracilis*. Our experiments on the organs of such animals as guinea-pigs, dogs, rats and pigeons show that various organisms occur constantly in cultures from the lungs, but are seldom found in cultures from the other organs, with the exception of the mesenteric lymph glands. In these *B. coli* and other bacteria sometimes occur. In cultures from the liver bacteria of intestinal origin as well as others are occasionally found. Cultures on agar from the livers of 16 out of 29 healthy guinea-pigs remained sterile, 6 showed one or two colonies of *B. subtilis*, 5 one or two colonies of cocci, and 1 two colonies and 1 several colonies of *B. coli*. Cultures from the livers of 5 dogs remained sterile. Cultures from the livers of 6 out of 8 rats remained sterile, 1 yielded two colonies of *B. subtilis* and 1 several colonies of *B. coli*. Cultures from the livers of 10 out of 11 pigeons remained sterile, while the other showed two colonies of *B. coli*. In spite of the fact that cultures on agar made with moderate quantities of liver tissue generally yield negative results we think that intestinal bacteria not infrequently gain entrance into the liver during life, and that positive results would be more frequently obtained if larger masses of the tissue and special media were used in cultivation. This view is borne out by the fact that large portions of liver tissue taken out of the body with every precaution and placed in sterile vessels rarely remain sterile, while portions of other organs, such as the kidney and spleen, usually do remain sterile.

Soon after death intestinal bacteria are found in considerable numbers in the liver and other organs. Cultures from the livers of guinea-pigs, rats and pigeons, made 24 hours after death, almost invariably produced numerous colonies of coli-like organisms.

According to Harden (1901) *B. coli* and allied organisms produce carbon-dioxide, hydrogen and nitrogen in varying quantities from glucose in the presence of peptone.

Chemical Changes in the Muscles and Organs.

As putrefaction proceeds the muscles lose their characteristic colour becoming pale pink, and later gray. They also lose their firm consistency, first becoming soft and easily detached from the bones, and later diffluent. The organs exhibit similar changes but at a more rapid rate than the muscles. Each of the various stages through which the muscles and organs pass is more or less recognizable with experience, but without a special nomenclature is difficult to describe in such a manner as to convey an intelligible picture to the reader.

It was found practically impossible to compare the disinfecting powers of different disinfectants with any degree of certainty by means of such methods. The possibilities of error were very great, and for purposes of comparison the results were lacking in precision. The elaboration of a new method, involving if possible definite figure comparisons, was therefore very desirable. We have obtained such figures by a method which enables us to estimate the amount of certain products present at any stage of the putrefaction.

The method is based upon the idea we entertained that the activity of putrefactive organisms depends upon the rapidity with which proteins and other complex organic substances present in the animal body are broken down into simpler substances, the latter constituting the real food of the organisms. Proteins for example are broken down into amino acids by means of proteolytic enzymes found in the organs and tissues of a body after death, and it can be easily shown that under suitable conditions putrefactive organisms destroy amino acids obtained in a tryptic digest with great rapidity. The ultimate nitrogenous products are ammonia or substituted ammonias of the volatile type. It is very difficult to conceive that organisms can break up the protein molecule without the assistance of proteolytic ferments, and we are inclined to the view that the organisms produce their own proteolytic ferments when they destroy enzyme-free protein. The more rapid

production of these bases in a tryptic digest may be accounted for if this view is correct. It follows from this that the true test of the power of a disinfectant would be determined by ascertaining to what extent it could prevent organisms from decomposing the amino acids of a tryptic digest. This would be best determined by estimating the proportion of amino acids to volatile bases in a proteolytic digest after incubation for a sufficient time. The enzymes responsible for the autolytic changes in the dead body of an animal are extremely resistant to many substances which kill the bacteria in cultures. In a carcase treated with a strong germicide the proteolytic enzymes of autolysis continue to act and produce amino acids, consequently the amino acids accumulate and chemical examination reveals a high ratio of amino acids to bases. Such a condition may be regarded as proof of the relative absence of bacterial action.

Changes due to autolytic enzymes.

In order to throw some light upon the relative rate of change in the various organs brought about by autolytic ferments after death, weighed portions of organs of a freshly killed dog were finely ground with sand in a mortar and triturated with water until three times as much water had been added. The liquids were then strained through muslin, and portions withdrawn, boiled to destroy their colour and filtered. A 10 c.c. portion of each filtrate was diluted, neutralised to phenol-phthalein, treated with neutral formaldehyde and titrated with N/10 soda according to the method of Sörensen. In order to inhibit bacterial action 1·5 % of toluene was added to the extracts, and they were incubated at 37° C. After certain periods portions were withdrawn, boiled, filtered and treated as before. The pancreas was diluted five times with water and the portions were not boiled before titrating. The following results were obtained:

TABLE VI.

Results of formyl titration in c.c. N/10 soda to neutralise.

	Immediate	1·75 hours	18 hours	24 hours	43 hours
Pancreas	1·2	3·7	9·6	10·7	10·7

	Immediate	17 hours	65 hours
Spleen	0·6	2·4	6·0
Liver	0·8	2·2	3·4
Kidney	0·6	1·8	2·9
Muscle	0·3	0·3	0·6*

* We are in doubt as to this figure, since shortly afterwards the culture was found to be contaminated with bacteria.

Except in muscle, a considerable development of substances containing the amino group in their constitution occurred. As a corresponding increase in acidity accompanied the increase in formyl titration the change may be attributed chiefly to the production of amino acids, the final products of the proteolytic ferments. Any ammonia which may be present is included in this titration, and it should be noted that relatively more ammonia in proportion to amino acids is produced in these digests than is produced by the action of trypsin on a protein. We attribute this higher proportion of ammonia chiefly to the action of the enzymes concerned with the breakdown of nucleic acid in the nucleo-proteins and nucleo-histones, which occur in such organs as the pancreas and lymphatic structures. This process results in the production of purine bases, guanine and adenine, either free or in the combined state as nucleosides. These in their turn are deaminised by the deaminases with the production of free ammonia. In the pancreatic digest the fermentation was very considerable and reached its maximum within 24 hours when incubated at 37° C. In another experiment four and a half to five days' incubation at 18° C. were required to reach the same titration value.

We have already noticed how slowly the muscles exhibit putrefactive changes. This fact is due probably to the lack of production of suitable conditions for putrefactive bacteria owing to the exceedingly slow rate of proteolytic change.

In order to ascertain the influence of aerobic and anaerobic conditions the following experiments were carried out. 100 grms. of the pancreas of a bullock, killed at 11·30 a.m., were ground up with sand at 3·0 p.m., and 250 c.c. of water, boiled for ten minutes to expel the dissolved oxygen and then cooled, added. The liquid was then lightly shaken, strained through muslin and 2 % of chloroform added. 5 c.c. portions were formyl titrated immediately, giving readings of 0·5 c.c. N/10 soda, and 10 c.c. portions distributed in wide mouthed test tubes arranged for anaerobic cultivation as described elsewhere (p. 143). A tube *A* with a cotton wool plug was kept as a control, and through the others various gases were passed for 3 minutes, and then the inlet tubes were sealed. The side tubes were filled with boiled, cooled water. Air was passed through tube *B*, oxygen through tube *C*, nitrogen through tube *D*, hydrogen through tube *E*, carbon dioxide through tube *F* and hydrogen sulphide through tube *G*. These tubes were incubated at 37° C., and daily a 5 c.c. portion from one of each series was formyl titrated.

TABLE VII.

Showing the influence of aerobic and anaerobic conditions on autolytic changes in the pancreas. The results are given in c.c. N/10 soda.

	18·5 hours	42 hours	66 hours
A. Control	1·9	3·0	3·35
B. Air	1·9	—	3·6
C. Oxygen	2·35	3·8	3·9
D. Nitrogen	1·8	3·05	3·6
E. Hydrogen	1·95	2·6	3·5
F. Carbon dioxide ...	1·45	2·65	3·45
G. Hydrogen sulphide	0·7	0·8	1·0

Oxygen increases the initial rate of production of substances responding to formyl titration. Within three days the contents of most of the other tubes had reached about the same level, somewhat below the level reached by the oxygen tube on the second day.

In a few hours the liquid in the hydrogen sulphide tube assumed a green tint, like that developed in the skin of a carcase after a few days' exposure, and it was found impossible to obtain a satisfactory titration without removing the hydrogen sulphide. For this purpose 5 c.c. of the contents of the tube were dried in a vacuum desiccator over potash, and then emulsified in water and titrated. It was found that the rate of action had been greatly retarded. A 5 c.c. portion after 66 hours' incubation was dried in vacuo and then emulsified with water and 2 % chloroform added. This fluid was incubated for 50 hours at 37° C. and titrated. It gave a reading of 1·35 c.c. N/10 soda. The presence of hydrogen sulphide to this extent therefore seems to inhibit enzyme action considerably. Judging by the reduction in titration to phenol-phthalein after drying there was not more than 0·112 % of hydrogen sulphide present.

Possibly the hydrogen sulphide produced by organisms in carcases has some inhibiting influence upon the action of autolytic enzymes.

The action of certain putrefactive organisms in pure culture have been studied to some extent, but the results obtained are of little value in elucidating the problems involved in putrefaction under natural conditions, since the consequences of enzyme action, of symbiotic association and of other important factors are not taken into consideration.

We desired to study the chemistry of putrefactive changes under conditions approximating as nearly as possible to those which occur in nature in order to throw some light on the phenomena we observed, and to help us in our selection of practical means for overcoming the nuisances occasioned by decomposing bodies. For this purpose two series of experiments were made in the following manner.

Methods of estimating chemical changes in cultures.

Series I. Four freshly killed guinea-pigs weighing 1502 grms. were skinned. The bladders and contents were removed, and the carcases

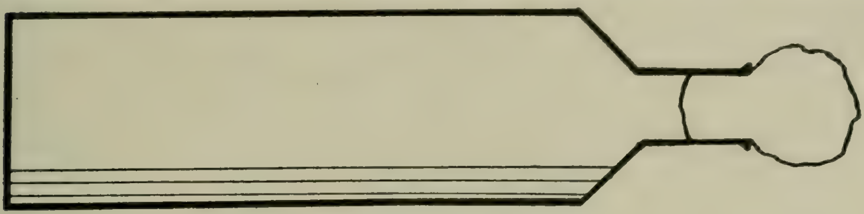

Fig. 2. Apparatus for aerobic cultivation.

and skins passed separately through a mincing machine. The whole of the mince so obtained was passed a second time through the mincing machine, and ground in a mortar. The mince was then thoroughly extracted with successive additions of water, all the fluid strained through muslin and finally passed through flannel by means of a press. Lastly the volume was made up to 1600 c.c. Then the fluid was divided into two equal parts to be cultivated aerobically and anaerobically. 25 c.c. portions were placed in flat medicine bottles loosely plugged with cotton wool (Fig. 2) for aerobic cultures, and 25 c.c. portions in large test tubes for anaerobic cultivation (Fig. 3). The test tubes were provided with air tight bungs pierced with two holes, one of which admitted a tube, passing to the bottom of the test tube, and the other a delivery tube, passing under N/10 hydrochloric acid contained in a second test tube open to the air. Before cultivation hydrogen was passed for 3 minutes through the tube first mentioned, which was sealed off after all the air had been displaced. By this means we permitted gases produced to escape, and provided against any loss of volatile bases.

Fig. 3. Apparatus for anaerobic cultivation.

Series II. Since it is generally held that putrefactive organisms gain entrance from

the intestines we decided to make a comparable series of experiments with carcases from which the whole intestinal tract from the oesophagus to the anus, together with the liver, had been removed between ligatures, so as to reduce as far as possible infection from its contents. The material, which consisted of the skins and bodies of six animals, weighing 1500 grms. was treated as previously described and the volume of the fluid made up to 1600 c.c. Both aerobic and anaerobic cultures were made.

Both series of cultures, aerobic and anaerobic, were cultivated at 27° C. and the aerobic bottles were placed on their sides to expose the greatest surface to the air, and were turned over daily.

The nitrogen present in 10 c.c. portions of the fluid first obtained in each series was estimated by Kjeldahl's method.

Series I. 10 c.c. contained 0·0587 grm. nitrogen corresponding to 0·9175 grm. of protein in 25 c.c. of fluid, calculated on the basis of 16 % nitrogen in protein.

Series II. 10 c.c. contained 0·0533 grm. corresponding to 0·832 grm. protein in 25 c.c. of fluid.

25 c.c. of the fluid from each series was taken immediately, 2 c.c. of water added, and made up to 250 c.c. with 97 % alcohol, and was allowed to stand for 24 hours by which means all the proteins, albumoses and peptones were completely precipitated. The precipitate, including the suspended matter, was filtered on tared paper, washed three or four times with 86 % alcohol and dried to constant weight at 100° C. The filtrate containing about 86 % alcohol holds in solution the amino acids and bases. To 100 c.c. of the filtrate excess of cold, saturated baryta solution was added (about 40 c.c.) and the volatile bases distilled over in vacuo at 40° C. into standard acid. The distillate was diluted to 500 c.c. with water and titrated with N/10 soda to methyl orange. The residue in the flask usually measuring about 10 c.c. was acidified whilst warm with hydrochloric acid, filtered and washed with water. To the filtrate, exactly neutralised to phenol-phthalein with soda, neutral formaldehyde was added and the amino acids titrated with N/10 soda according to Sörensen's method.

Every day aerobic and anaerobic cultures of both series were treated in the way just described. The apparatus used is shown in Fig. 4.

The organic acids volatile in steam were estimated in some cases by removing the volatile bases in the way described, acidulating the residue in the flask with dilute sulphuric acid, and distilling with steam and titrating with N/10 soda to phenol-phthalein.

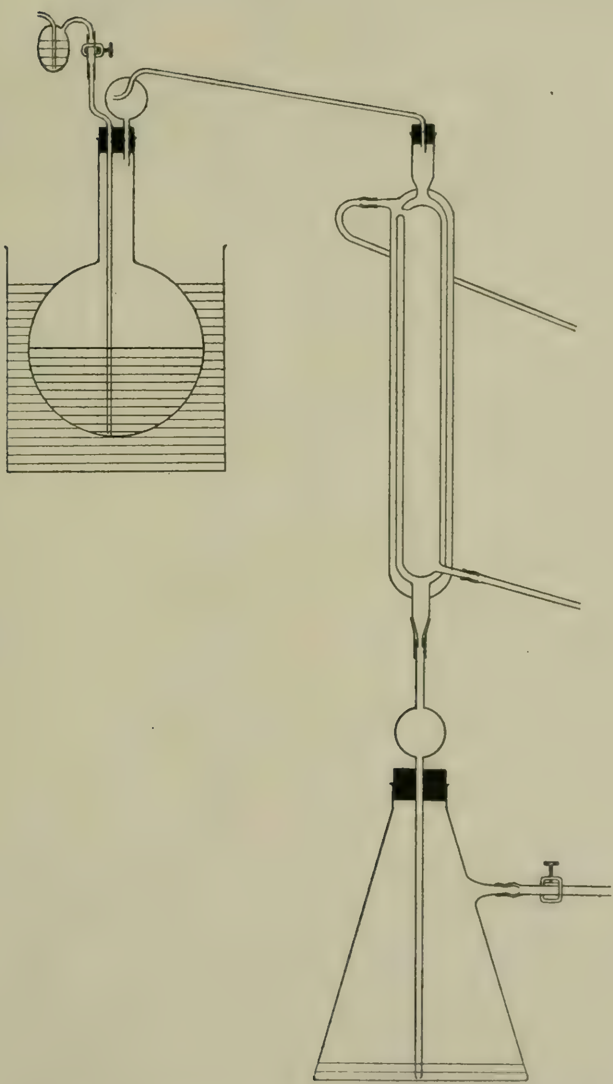

Fig. 4. Apparatus for the estimation of volatile bases.

TABLE VIII.

Showing the results of the analyses of the cultures. Intestines included.
(Series I.)

	Aerobic				Anaerobic			
		in two-fifths of culture				in two-fifths of culture		
Days	Grms dry matter insol. in 86 % alcohol	Volatile bases c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids	Grms dry matter insol. in 86 % alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
Orig.	0·9856	2·3	1·75	1·3 : 1	0·9856	2·3	1·75	1·3 : 1
1	0·9220	4·05	1·4	2·9 : 1	0·8310	4·7	3·0	1·6 : 1
2	0·7619	12·85	1·15	11·2 : 1	0·7764	6·2	3·7	1·7 : 1
3	0·6461	18·35	1·3	14·1 : 1	0·7495	8·4	4·15	2·0 : 1
4	0·5857	21·4	1·1	19·4 : 1	0·6259	14·45	4·25	3·4 : 1
5	0·5554	23·9	0·85	28·1 : 1	0·5010	21·0	3·7	5·6 : 1
6	0·5240	25·6	0·5	—	0·5075	—	4·75	—
7	0·5288	25·1	0·35	—	0·4335	22·45	4·3	5·2 : 1
8	0·5244	—	—	—	0·4442	—	—	—
9	0·5186	24·1	0·3	—	0·3905	25·9	4·6	5·2 : 1
10	0·5225	—	—	—	0·3737	—	—	—
11	0·5220	24·35	0·4	—	0·3689	25·5	4·8	5·4 : 1
12	0·5231	—	—	—	0·3309	27·7	—	—
13	0·4915	24·15	0·2	—	0·3165	28·15	4·0	7·0 : 1
14	0·4957	—	—	—	0·3166	—	—	—
15	0·4900	—	—	—	0·3922	—	—	—
16	0·4627	18·8	0·0	—	0·2587	—	—	—
17	0·4712	19·85	0·0	—	0·2614	—	—	—
18	0·4876	—	—	—	0·2857	—	—	—
19	0·4817	—	—	—	0·2781	—	—	—
20	0·4655	—	—	—	0·2580	—	—	—
21	0·4457	15·15	0·0	—	—	—	—	—
22	0·5036	—	—	—	0·2747	—	—	—
23	0·4621	12·1	—	—	0·2508	30·95	—	—
24	0·4622	—	—	—	0·2385	—	—	—

The larger quantity of dry matter in series I is probably accounted for by the inclusion of intestinal contents. In the aerobic cultures of both series only a slight fall in dry matter occurs on the first day, but subsequently the fall is rapid till a more or less constant level, approximately one half of the original, is reached on the sixth day. The disappearance of dry matter is greatest on the second day. This reduction in weight of dry matter is less than the increase in bases represents when calculated into terms of protein at 16 % nitrogen. Since arginine is said to be absent from the products of autolysis the high yield of bases relative to dry matter consumed may be due partly to the rapid action of arginase splitting off urea from the arginine molecule, and the subsequent rapid deamination of the urea.

TABLE IX.

Showing the results of the analyses of the cultures. Intestines excluded. (Series II.)

	Aerobic				Anaerobic			
		in two-fifths of culture				in two-fifths of culture		
Days	Grms. dry matter insol. in 86 % alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids	Grms. dry matter insol. in 86 % alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
Orig.	0·6802	0·7	1·25	0·56 : 1	0·6802	0·7	1·25	0·56 : 1
1	0·6535	3·4	0·65	5·2 : 1	0·6353	2·1	1·4	1·5 : 1
2	0·5340	7·55	1·2	6·3 : 1	0·5930	5·85	1·5	3·9 : 1
3	0·4574	10·9	0·85	12·8 : 1	0·5529	7·31	1·45	5·0 : 1
4	0·3766	14·15	1·05	13·5 : 1	0·5143	9·3	1·5	6·2 : 1
5	0·3484	15·85	1·0	15·85 : 1	0·4459	13·9	1·25	11·1 : 1
6	0·3262	15·85	1·0	15·85 : 1	0·4063	14·85	1·25	11·9 : 1
7	0·3336	15·8	0·9	17·6 : 1	0·3379	17·25	1·35	12·8 : 1
8	0·3261	14·95	1·05	14·2 : 1	0·3516	—	—	—
9	0·3117	—	—	—	0·3718	15·25	1·25	12·1 : 1
10	0·3281	16·3	0·3	—	0·2837	—	—	—
11	0·3245	16·1	0·15	—	0·2553	20·75	1·55	13·4 : 1
12	0·2935	17·1	0·0	—	0·2572	—	—	—
13	0·3195	—	—	—	0·2942	—	—	—
14	0·2897	—	—	—	0·2741	20·25	1·2	16·8 : 1
15	0·3263	—	—	—	0·2580	—	—	—
16	0·3108	13·65	0·1	—	0·2496	21·5	1·3	16·5 : 1
17	0·2838	13·4	0·0	—	0·2343	—	—	—
18	0·3010	12·4	0·0	—	0·2086	22·75	1·5	16·1 : 1
19	0·2850	12·75	0·05	—	—	—	—	—

In the anaerobic cultures the reduction of dry matter continued and had not reached a constant level at the end of three weeks. By this time about 75 % in series I and 66 % in series II had disappeared. In the anaerobic cultures of series I the highest reduction is on the first day, though the production of bases was only slightly greater than in the aerobic series. On this day the non-nitrogenous constituents, probably the carbohydrates in the intestinal contents, were attacked, as the bases correspond to only 5·4 % nitrogen in the dry matter which has disappeared. In the aerobic cultures of series II, in which the intestinal contents were absent, this phenomenon was not so evident.

In the aerobic and anaerobic cultures of both series the base production corresponded roughly with the disappearance of dry matter. If, however, the amount of nitrogen present in the products be calculated each day as percentage of nitrogen in the corresponding dry matter lost considerable daily variation is shown. On the second and third days in the anaerobic cultures of series I and II a very high figure is

obtained. This suggests the decomposition of bodies such as creatin and urea, which contain high percentages of nitrogen. On the sixth day in both series a very low percentage in the dry matter lost is seen. Such variations might be expected when the complexity of the culture medium is taken into consideration. An estimation of the disappearance of dry matter alone would fail to bring out these points.

In the aerobic cultures of both series the substances which respond to the formyl titration show little change for the first week. In both cases a rapid fall occurs afterwards, and after a fortnight little, if any, of these substances remain. The volatile bases begin to decrease when no amino acids are left. This decrease cannot be accounted for by volatilization during incubation. Under the conditions of these experiments putrefaction seemed to cease about this time. A very great number of factors may be concerned in this phenomenon, but we should like to point out that the activity of the aerobic bacteria especially may depend on the presence of preformed products of autolysis. The disappearance of the bases suggests that when the amino acids have been used organisms seek another source for their nitrogen requirements[1].

In the anaerobic cultures of both series on the other hand putrefaction proceeded throughout the whole period of observation as evidenced by the decrease in dry matter and the increase in bases. In series I the formyl titration increased till the third day and subsequently remained nearly constant, but in series II it remained practically constant throughout, but at a much lower level. Presumably the greater formyl titration in series I is due to the inclusion of the intestinal contents. We have not determined to which of the amino acids the phenomenon of constancy may be attributed, but we have confirmed some of our figures by van Slyke's nitrous acid method.

In order to eliminate the non-spore-bearing organisms 25 c.c. portions of the original fluid used in series II were heated to 80° C. for 20 minutes, and incubated aerobically and anaerobically at 27° C. for 21 days.

TABLE X.

		In two fifths of culture	
	Dry matter used in 80 % alcohol	Volatile bases c.c. N/10 acid neutralised	Formyl titration N/10 soda
Aerobic ...	0·3288	5·8	0·0
Anaerobic ...	0·1537	24·95	1·7

[1] It is interesting to consider these results in relation to the losses of nitrogen which occur in farmyard manure under aerobic conditions.

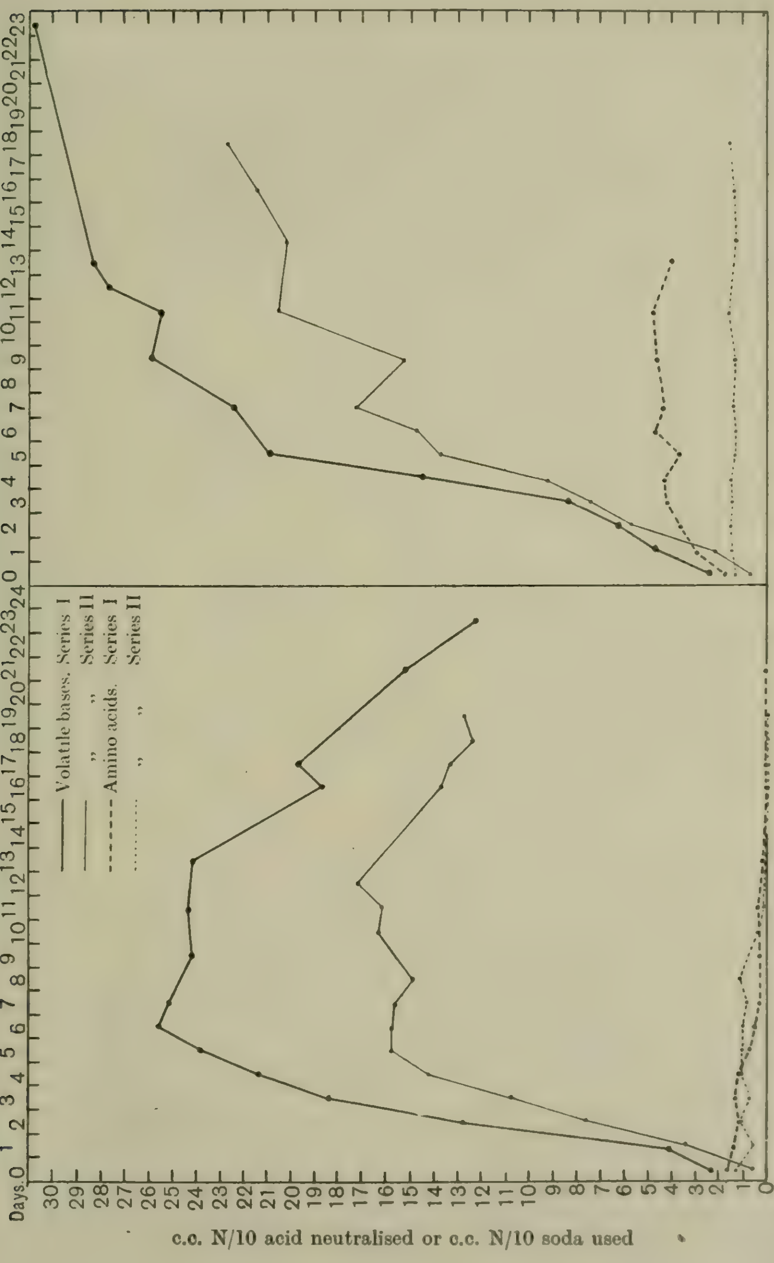

Chart 2. Showing the quantities of volatile bases and amino acids present daily in the aerobic and anaerobic cultures of series I and II.

In the aerobic culture the disappearance of bases is very evident. Possibly the aerobic spore-bearing organisms are responsible for this disappearance. An anaerobic culture of series II incubated for 21 days would have given similar figures to the culture incubated anaerobically after heating. The inference is that in both the changes have been brought about by anaerobic spore-bearing organisms.

In a preliminary experiment with cultures of series II the following figures for organic acids volatile in steam were obtained, after deducting 5·25 c.c. N/10 soda (due to the presence of carbonate in the soda and baryta used), the figure found in a "blank" experiment.

TABLE XI.

Organic acids volatile in steam in the cultures of series II.

Incubation period Days	Aerobic cultures c.c. N/10 soda required	Anaerobic cultures c.c. N/10 soda required
Original	7·75	7·75
1	7·0	2·05
2	10·25	7·25
3	—	6·6
4	23·85	31·65
5	7·45	10·85
6	2·2	17·80
7	1·0	10·65
8	15·1	—
9	0·35	17·75
10	—	—
11	17·65	—
12	2·45	29·95
13	4·7	—
14	23·25	—
15	—	17·85
16	8·75	—
17	—	—
18	—	—
19	—	—
20	1·25	19·55
Cult. originally heated to 80° C. 21st day	20·1	23·05

Chart 3 and Table XI show that there are great daily variations in the quantities of these acids present indicating that there are several phases in the breakdown of the materials in the cultures. More determinations would be required before the figures could be satisfactorily interpreted. We have been unable to investigate this matter further.

The following quotation from Rettger (1906, p. 81) illustrates some of the difficulties associated with the investigation of putrefaction. "When the obligate anaerobes were cultivated along with other bacteria decidedly different results were obtained and here again my observations support those of Bienstock. The rate and nature of decomposition depend on the particular kinds of organisms with which the anaerobes were mixed. The *B. coli communis* and *B. lactis aerogenes* actually

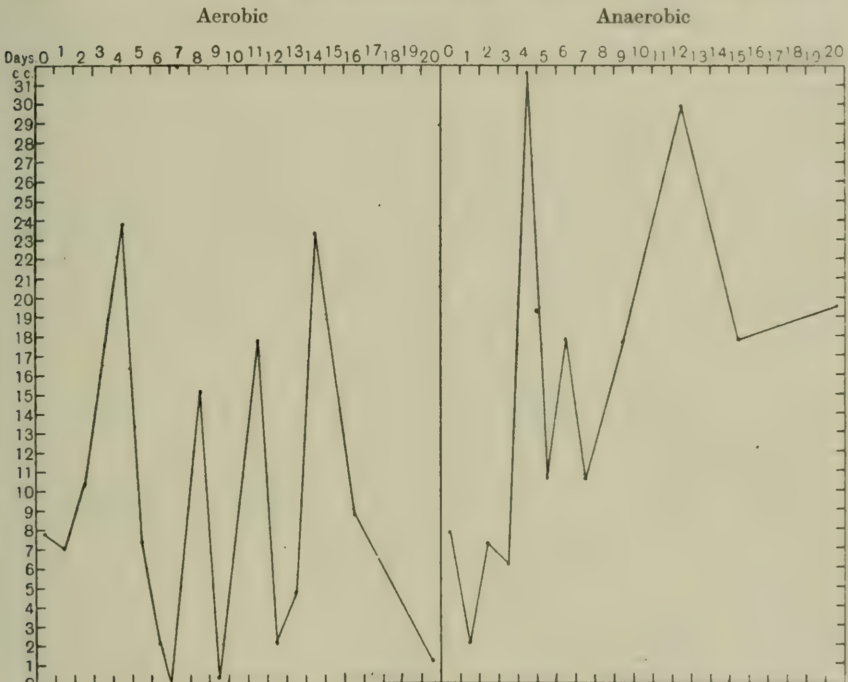

Chart 3. Showing the quantities of N/10 soda neutralised by organic acids volatile in steam in aerobic and anaerobic cultures of series II.

checked the rate of decomposition in egg-meat mixture and hence caused a decided decrease in the amount of certain products in the given length of time....In fact this antagonism of the colon bacillus to the anaerobe was most noticeable at all times and in every experiment. On the other hand the action of *Proteus vulgaris* was most favourable to the rapid disintegrative action of the anaerobes."

In such experiments as we have quoted slight errors from various causes are unavoidable. It is difficult for example to obtain absolute uniformity in the composition of the fluid placed in each culture tube,

various amounts of solid matter stick to the sides of the vessels above the fluid during incubation, etc.

Methods of estimating the chemical changes in fluids draining from carcases.

Passing from experiments with finely divided animal remains we may consider the results obtained by applying the same methods to carcases.

In order to determine the changes exhibited daily by the fluid draining from a carcase the body of a guinea-pig, which had died from natural causes, was placed in a large bottle with a loosely fitting cork, and kept at 37° C. On the second day 52·5 c.c. of fluid, almost free from sediment, had drained from the body and was pipetted out of the bottle. The fluid was filtered and 5 c.c., mixed with 22 c.c. of water, placed in a flask and made up to 250 c.c. with 97 % alcohol. Every subsequent day the fluid was removed from the bottle and measured, and a portion treated in the way described. The alcohol extracts were analysed in the manner described on p. 144.

TABLE XII.

Showing the changes exhibited daily in fluid draining from a carcase.

			In two-fifths of sample		
Days	c.c. of fluid collected	Grms dry matter insol in 86 % alcohol in 5 c.c.	Volatile bases c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
2	52·5	0·207	5·3	2·3	2·3 : 1
3	21·0	0·2194	8·6	2·6	3·3 : 1
4	23·0	0·2156	13·0	2·3	5·6 : 1
5	9·0	0·1866	16·05	2·2	7·3 : 1
6	4·5	—	—	—	—
7	5·0	—	—	—	—
8	5·0	0·1117	22·3	2·3	9·8 : 1
9	6·0	0·0905	—	—	—
10	6·0	0·084	26·2	2·8	9·3 : 1
11	6·0	0·066	—	—	—
12	5·5	0·0712	29·9	2·3	12·9 : 1
13	2·5	—	—	—	—
14	4·25	0·0656	—	—	—
15	5·5	—	—	—	—
16	1·5	—	—	—	—
17	3·0	—	—	—	—
18, 19	5·0	—	—	—	—
20, 21	5·0	—	34·9	2·75	12·7 : 1
	170·25				

The original weight of the animal was 404·5 grms., and the remains of the carcase on the 21st day weighed 198 grms., and after complete maceration, removal of fat and drying the bones weighed 19·25 grms.

We see from this experiment that an analysis of the fluid draining from the body yields evidence of progressive putrefactive changes, similar in all respects to those occurring in the anaerobic cultures of series II. The experiment also shows that the products of putrefaction are continually draining away from the carcase. This occurs in the exposed body.

We may next quote the results of our analyses of the fluids which drained from the carcases used in the second gas experiment (p. 130), and compare them with the results of the analyses of portions of muscle. In each case a muscle dissected from the thigh was taken, and the larger masses of connective tissue removed. The remainder was weighed, ground to a paste with sand, triturated with a little water, and the fluid made up to a definite volume with 97 % alcohol. In each case sufficient water was used to bring the final concentration of alcohol to 86 %.

TABLE XIII.

Analyses of the fluids taken from the bottles on the 21st day.

Guinea-pig (p. 130)	Original weight of carcase	c.c. of fluid collected	Grms. dry matter insol. in 86 % alcohol in 5 c.c.	In two-fifths of sample: Volatile bases. c.c. N/10 acid neutralised*	In two-fifths of sample: Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
J	272	86	0·021	17·3	1·7	10·1 : 1
K	305	50	0·148	26·4	2·85	9·2 : 1
M	147	20†	0·164	26·0	2·47	10·5 : 1
L	295	30†	0·114	25·9	1·87	13·9 : 1
O	202	45	0·058	16·7	2·1	8·0 : 1
Q	316	46	0·082	26·2	2·55	10·3 : 1
S	380	60	0·133	17·1	2·5	6·8 : 1
R	387	45	0·092	6·2	5·6	1·1 : 1
N	284	53	0·203	8·3	5·7	1·4 : 1
T	17	5·5	0·127	20·3	3·1	6·5 : 1

* To make certain that the bases or other constituents of creosote oil were not influencing these figures 10 c.c. of creosote oil were treated with 53 c.c. of water (*Note.* Guinea-pig *N* treated with 10 c.c. of creosote oil gave 53 c.c. of fluid) and shaken occasionally during 26 days. The creosote oil was then filtered off and the filtrate again filtered. 40 c.c. of the clear filtrate was analysed in the same way as the body fluid. The volatile bases, presumably ammonia, neutralised 1·0 c.c. N/10 acid and the filtrate gave no formyl titration. As the fluid which exuded from the carcases was filtered and the equivalent of 2 c.c. analysed the presence of creosote oil could not influence the formyl titration, and could not increase the reading for volatile bases by more than 0·05 c.c. N/10 acid.

† Owing to the condition of these carcases (p. 132) it was impossible to be certain that all the fluid had been collected. It should be noted that in *L* and *M* blood escaped before the carcase was placed in the bottle.

While the figures for the volatile bases give an excellent indication of the condition of the carcase the figures in the ratio column place the carcases in nearly the same order as that indicated by the dissections (p. 133), and have the great advantage over descriptions of showing the degree of putrefaction in numerical terms. On referring to the tables of the anaerobic cultures of series I and II it will be seen that putrefaction in carcases treated with creosote oil has only advanced to a very slight extent, equivalent to the stage reached on the first day in the cultures. The much higher content of amino acids in the fluids in these carcases shows that the activity of putrefactive organisms has been reduced to such an extent as to be negligible from the practical point of view. Even the bases found in them may be due largely to ammonia produced by autolytic enzymes and not to bacterial action.

In the carcases with the skins treated with "5 % cresols' emulsion" putrefaction had not advanced so far as in the untreated carcases, but in the injected one very little inhibition of putrefaction had occurred. Owing to an accident no figures for the carcase injected with creosote oil were obtained, but it seemed as much decomposed as that injected with cresols' emulsion.

We believe that this method of determining the ratio of volatile bases to the amino acids will be found to constitute a reliable means of ascertaining the extent to which putrefaction has advanced.

The analyses of the muscles yielded results differing only in degree from the results obtained in the fluids as may be seen by reference to Table XIV.

TABLE XIV.

Analyses of muscles.

Guinea pig	Weight of muscle taken	Grms dry matter insol in 80 % alcohol per grm muscle	Volatile bases per grm muscle N/10 acid neutralised	Amino acids per grm muscle N/10 soda	Volatile bases + amino acids per grm. muscle	Ratio of volatile bases to amino acids
J	2·260	0·2157*	10·29	1·60	11·89	6·42 : 1
K	1·718	0·1522	10·77	1·31	12·08	8·22 : 1
O	2·833	0·1374	7·94	0·97	8·91	8·18 : 1
S	3·505	0·1514	8·10	1·07	9·17	7·57 : 1
N	2·779	0·2346	2·87	0·72	3·59	3·94 : 1
R	5·819	0·1921	3·22	1·89	5·11	1·70 : 1
Control fresh	3·522	0·2939	0·35	0·21	0·56	1·66 : 1
Control 24 hours at 22° C.	3·511	0·2774	0·39	0·36	0·75	1·10 : 1

* In this case less of the connective tissue was removed from the muscle before grinding than in the others.

In this table analyses of muscles from a freshly killed guinea-pig and from a body which had been kept for 24 hours at 22° C. have been inserted for the sake of comparison.

Table XIV places the carcases (except *J*) in the same order as Table XIII, but shows that putrefaction had advanced further in the carcase with its skin treated with creosote oil (*N*) than in the one in which the skin and peritoneal surfaces had been treated (*R*). This apparent discrepancy may perhaps be explicable in the following way.

The fluid which exudes, charged with the products of changes occurring in the body, after passing through the skin treated with the strong antiseptic, creosote oil, remains in the bottle without undergoing further changes. The greater part of the fluid is exuded in the first few days, and later, perhaps owing to the action of organisms derived from the untreated intestines, some of the amino acids in the muscles are converted into bases, causing the differences noticed between analyses of the fluids and muscles on the 21st day.

We have then several methods of ascertaining the degree of putrefaction, and for the sake of comparison we have compiled Table XV which shows at a glance the values attached to each method.

TABLE XV.

		Result of dissection	Total gas per grm. in c.c.	Ratio in fluid	Ratio in muscle
R	Skin and peritoneal surfaces treated with creosote oil	well preserved	4·51	1·1 : 1	1·70 : 1
N	Skin treated with creosote oil ...	,, ,,	6·02	1·4 : 1	3·94 : 1
S	Skin and peritoneal surfaces treated with "5 % cresols"	putrid	5·69	6·8 : 1	7·57 : 1
J	Intact carcase	,,	7·82	10·1 : 1	6·42 : 1
K	Abdominal cavity opened	very putrid	7·36	9·2 : 1	8·22 : 1
L	Bled	,,	10·24	13·9 : 1	—
M	Skin and abdominal organs removed	,,	9·45	10·5 : 1	—
O	Skin treated with "5 % cresols" ...	,,	8·44	8·0 : 1	8·18 : 1
P	Injected with creosote oil	,,	8·3	—	—
Q	Injected with "5 % cresols" ...	,,	8·66	10·3 : 1	—

The necessity for applying ourselves to the more practical aspects of the problem prevented us from developing the experiments described in the last two sections sufficiently to work out some of the more important considerations which they suggest. By slight modifications in the procedures and the form of the apparatus it should be possible to ascertain the precise effects of the draining away of the body fluids, the importance of the skin and various organs, the effects of the food eaten before death, the actions of the enzymes and of different groups

of organisms, alone and in combination, on the various constituents of the body, the conditions which govern their actions, the nature, origin, rate of production and significance of the gaseous products and the effects of antiseptics, applied in various ways.

We need hardly point out how important might be the effects of an accurate knowledge of the factors which govern putrefaction. Changes of a putrefactive nature in the intestine probably exert a great influence on health, and their presence and extent could be ascertained. The benefits to those engaged in work connected with medicine, sanitation, animal nutrition, meat preservation and allied problems would be immense.

Putrefactive Odours.

The stench from a decaying carcase is a combination of odours, and varies in character at different times. At various stages the predominance of one constituent over others can be distinguished. In the earlier stages hydrogen sulphide can be recognized, later such bodies as methylmercaptan, still later, when liquefaction is in progress, the amines. The stenches change in character with changes in the environment. Some of the odoriferous substances are more soluble than others, and the action of water is liable to mask certain odours, and causes others to predominate. A rancid odour due to organic acids of the butyric type can be detected in some cases.

We have found it impossible to give an intelligible description of the odours arising in the later stages of putrefaction. We can, however, obtain some conception of the odours by dividing them into their main groups, namely those arising from organic bases, from organic acids and from sulphur compounds.

The presence in a putrefying fluid of odours arising from organic bases can be demonstrated by adding alkalis to fix the free organic acids, and of those from organic acids by adding sufficient mineral acid to combine with the basic constituents.

A perfect deodorant should contain chemical substances capable of eliminating all the constituents, which go to make up the stench. Deodorants of a purely acid nature can fix only the bases, while setting free the organic acids responsible for the rancid odours. In like manner basic deodorants fix organic acids and set free the bases. On the other hand some deodorants, such as oxidising agents, may destroy substances giving rise to odours and not merely fix them. Chemical action resulting in such destruction may be facilitated by the deodorant containing

solvents for constituents of the odour. Some deodorants only dissolve certain noxious substances and hold them in solution for longer or shorter periods according to their rate of evaporation.

The period during which a deodorant remains operative depends to a large extent on its rate of evaporation, degree of solubility in water, and its power of stopping putrefactive changes in the substances with which it is in contact. In estimating the action of a deodorant it must be remembered that some substances used for this purpose affect the nasal mucous membrane.

By the time we commenced to investigate the stenches arising from putrefying material we had been greatly impressed by the deodorising properties of creosote oil and similar bodies. Nevertheless we decided to compare the results obtained with agents of different types reputed to be efficient, as well as with others, which from their chemical nature might prove of value. We experimented with oxidising agents of varying power and with acids and bases, alone and in combination. Powerful oxidising agents like bleaching powder and potassium permanganate are efficient for a short period, when applied in such quantities as can be used in practice, but since putrefying materials contain large quantities of reducing substances their action is evanescent. Weaker oxidising agents such as potassium bichromate are not so efficient at first, but not being so easily reduced they exert an action for a longer time. Potassium bichromate alone removes many of the odours, except those of a rancid type. The addition of milk of lime removes these also. As potassium bichromate acts more powerfully as an oxidising agent in the presence of acid we have tested this combination, which includes the additional effect of the acids on the basic substances responsible for some odours.

We quote in detail a few only of our experiments for the purpose of illustrating the effects of some of the deodorising substances we have mentioned.

In the first series of experiments we placed portions, weighing approximately 5 grms., of disintegrating intestine from a rabbit in an advanced stage of decomposition in small beakers and treated them with 5 c.c. of the various solutions or emulsions. Observers ignorant of the contents and treatment checked our results. The type of stench differed from that of a putrefying carcase being considerably less basic in character.

The odours in the beakers numbered 2, 3, 4, 5, 6, 8 and 10 did not differ materially from the control No. 1 at any time. In those numbered 7, 9, 11 the stench was obliterated at first, but was as bad as in the

TABLE XVI.

Showing the effects of various reagents on putrefactive odours.

	Reagent added to material	15 mins	60 mins	24 hrs	48 hrs	72 hrs	9 days
1.	Water (control)	—	+ + +	+ + +	+ + +	+ + +	+ + +
2.	Mercuric chloride 0·104 %, hydrochloric acid 0·208 % + aniline blue 0·04 %	+	+	+ + +	+ + +	+ + +	+ + +
3.	Mercuric chloride 0·104 %, hydrochloric acid 0·208 % ...	+ +	+ +	+ + +	+ + +	+ + +	+ + +
4.	Aniline blue 0·04 %	+	+ + +	+ + +	+ + +	+ + +	+ + +
5.	Hydrochloric acid 1 %	+	+ + +	+ + +	+ + +	+ + +	+ + +
6.	Soda 0·5 %	—	—	+ +	+ +	+ + +	+ +
7.	Bleaching powder 1 %	✻	0	+	+ +	+ +	+ + +
8.	" " 0·1 % ...	+	+ +	+ +	+ +	+ + +	+ + +
9.	" " 1 % + boric acid 0·5 %	0	0	✻	+ +	+	+ +
10.	Bleaching powder 0·1 % + boric acid 0·5 %	+	+ +	+ +	+ + +	+ + +	+
11.	Potassium permanganate 1 %	0	✻	+ +	+ + +	+ + +	+ +
12.	" bichromate 1 % ...	+	✻	0 ?	✻	—	—
13.	" " 1 % + boric acid 1 %	—	—	✻	✻	0 ?	0
14.	Potassium bichromate 1 % + hydrochloric acid 1 % ...	—	—	✻	✻	0 ?	0
15.	Crude carbolic acid 1 % ...	+ +	+ +	✻	✻	0 ?	0
16.	Phenol 1 %	+	+ + +	+ + +	+	+ +	+
17.	Creosote oil 5 %	+	✻	0	0	0	0

0 indicates no odour beyond that of the reagent, ✻ slight smell, + very distinct putrescent odour, + + more marked, + + + almost intolerable stench, — not tested.

control within 24–48 hours. In No. 16 there was some diminution in the stench after 48 hours. In No. 15 the stench diminished in 24 hours and ultimately disappeared. In Nos. 12, 13, 14, 17 a great reduction in stench was very soon apparent, and complete and permanent deodorisation occurred after 24 hours.

In the second series of experiments the much decomposed carcases of guinea-pigs were placed in bottles, and 50 c.c. of the reagent added. In one set the bottles were corked and in the other open.

TABLE XVII.

Showing the effects of certain reagents on putrefactive odours.

	Corked			Open		
Reagent	17 hrs	48 hrs	8 days	17 hrs	48 hrs	8 days
Bleaching powder 2 %	0	+	+ + +	+	+ +	+
Bleaching powder + boric acid 1 % ...	+	+ + +	+ + +	+ +	+ +	+
Potassium bichromate 2 %	0	✻	✻	0	✻	✻
Potassium bichromate 2 % + aniline blue 0·1 %	0	✻	✻	0	✻	✻
Crude carbolic acid 2 %	0	0	✻	0	0	+

In the 3rd, 4th and 5th series of experiments a mince made from beef and kept for two, three and eight days respectively at 37° C. was employed. In all the experiments the stench was horrible, but its character was different in each experiment. In each case 5 grms. of the mince was used, and 5 drops of the reagent run over it, and the beakers carefully examined within an hour.

TABLE XVIII.

Showing the effects of various reagents on putrefactive odours.

Reagent	Experiment 3	Experiment 4	Experiment 5
Water	+ + +	+ + +	+ + +
"Cresols' emulsion 5 %" ...	+ + +	+ + +	+ + +
Creosote oil	*	0	0
Creosote oil + bone oil 3 % ...	0	0	0

It was noticed in the course of these experiments that the addition of water robbed the stench of some of its constituents, and that the addition of an equivalent quantity of 5 % cresols' emulsion acted in the same manner and approximately to the same extent.

Taking into consideration the differences in the materials to be deodorised the results agree with those of series I. The bleaching powder produces a temporary effect, the potassium bichromate a beneficial effect for a considerable time, and crude carbolic (2 %), while efficient at first, permits of the development of odour later. Again creosote oil and its emulsions give the most satisfactory results.

Finding that emulsions of certain crude tar oils yielded excellent results we next proceeded to examine the deodorising properties of undiluted coal-tar oils, and of products obtained by their distillation. The latter part of the work was carried out in order to determine the actions of the groups of constituents, and of some component parts of the groups. For example the first fraction of creosote oil, distilling at 170–220° C. and representing 28 % of the oil, was separated into its three principal constituents, (*a*) the tar acids or phenolic bodies, (*b*) the bases, and (*c*) the hydrocarbons. The groups (*a*) and (*c*) were subdivided by fractional distillation. In the group (*b*) an "insoluble" oil was separated by treating the acid extract with soda and a "water soluble" part by further extraction of the fluid with ether.

The experiments were carried out in the same manner as those just quoted, using 10 grms. of a mixture of equal parts of meat and intestinal contents from a decaying body. 10 drops of the reagent were run over the surface of the material to be treated.

TABLE XIX.

Showing the effects of tar oils and certain of their constituents on putrefactive odours.

	10 mins.	45 mins.	20 hrs.
Crude tar oils.			
Crude carbolic acid	+	0	0
"Middle oil"	*	0	0
Creosote oil	*	0	0
"Heavy oil"	*	+	0
Anthracene oil	+ +	*	0
Fractions of creosote oil.			
170—220° C.	*	0	+
220—240° C.	*	0	+
Residue	*	0	*
Fractions of the 3 groups of constituents from the fraction 170—220° C. of creosote oil.			
Phenolic bodies, complete mixture of	*	+ +	+
Fraction 77—191° C.	0	0	0
„ 191—200° C.	0	+	*
„ 200—210° C.	*	+	+
Residue	+	+	*
Bases—"water soluble fraction"	0	0	0
"water insoluble fraction"	0	0	0
Hydrocarbons.—Complete mixture	0	0	0
Fraction 80—170° C.	0	0	0
„ 170—180° C.	0	0	*
„ 180—190° C.	0	0	0
„ 190—200° C.	0	0	0
„ 200—210° C.	0	0	0
„ 210—225° C.	0	*	+
„ 225—240° C.	0	*	0
Residue	0	*	*
"Calcium cresolate" *	+ +	+ +	+
Water extract of creosote oil †	+ + +	+ + +	+
Control	+ + +	+ + +	+ + +

* Extracted from creosote oil by milk of lime; equivalent to 0·91 % calculated as cresols.

† Equivalent to 0·62 % cresols.

We also carried out experiments with substances obtained by fractional distillation of crude carbolic and "middle oils." In the former the fractions which came off up to 187° C. were the more efficient, and in the latter those which came off up to 210° C.

All the crude tar oils produced complete deodorisation in 20 hours, but the "heavy oil" and the anthracene oil took longest to produce this result. We have recorded in Table XIX the impressions of several

observers, who were ignorant of the objects of these experiments. Each of the substances used has its own characteristic odour, which tends to distract attention from the odours arising from the material to be tested. Also some of these characteristic odours seem to affect the nasal mucous membrane to a slight extent for shorter or longer periods. Bearing these facts in mind the observers smelt the beakers with the very greatest care, and if at any time an odour other than that of the reagent was noticed, the fact was recorded. Some of the reagents possess distinctly unpleasant odours and are therefore unsuitable for practical use, but this is not the case with creosote oil, which has a not unpleasant odour. The first fractions of creosote oil undoubtedly contain small quantities of the substances, such as thiophene, which impart the characteristic smell to crude carbolic acid and "middle oils," but their rate of evaporation is retarded by mixture with the other constituents, and hence they do not make their presence evident in whole creosote oil. Consequently the deodorising properties of creosote oil can be more easily determined than those of other coal-tar oils. Taking into consideration its power of stopping putrefaction, of killing maggots, its deodorising properties and pleasant smell we regard creosote oil as the most suitable reagent to employ as a deodorant[1].

We were obliged to relinquish our investigations on the stenches arising from decaying bodies and the methods of dealing with them after we had reached the conclusions just recorded. It is evident however that further research on this very important subject could be undertaken with great advantage.

FLUIDS EXUDING FROM CARCASES.

Mention has been made of the fluid which exudes from the carcase, first appearing in an area on the left side of the body in a small animal. Some experiments were undertaken with the object of ascertaining the origin of the fluid, the factors which determine the locality in which it first appears, and the influence of antiseptic reagents on its production.

The carcases of five guinea-pigs were kept in a dry atmosphere at a temperature of 60° F. in order to eliminate the influence of moisture, thus giving a better opportunity of observing accurately the sequence of events. One carcase was laid on its ventral surface, one on its right

[1] As phenol and its homologues are extremely weak acids, weaker even than carbonic acid, the bases in creosote oil although present in small quantities relative to the phenolic bodies are available to exercise their influence upon the acid constituents of stenches when creosote oil is used as a deodorant.

side, one on its left side, one on its dorsal surface and one was suspended by its head. In all cases the fluid first appeared in a small area situated on the left side below the ribs. The direction of spread depended to some extent on gravity, but in all cases the superficial epidermis over nearly the whole body was loose and moist by the 7th day. On the 8th day the carcases were dissected under water. All were in the same condition as the body described on p. 126. Thinking that the digestive or autolytic ferments were in some way concerned with this phenomenon we carried out the following series of experiments. Immediately after death the body was opened by a median incision, organs excised with precautions to avoid contamination as far as possible, and the body sewn up by a double layer of sutures, one through the abdominal muscles and the other through the skin. That this procedure was efficient in preventing gas from escaping is shown by the fact that many of the carcases became greatly distended with gas.

TABLE XX.

Showing the influence of the abdominal organs on the exudation of fluid.

1.	Stomach alone removed	Fluid appeared in the usual place on 5th day		
2.	Stomach ligatured at both ends, but left *in situ*	„	„	„
3.	Stomach and intestines removed ...	„	„	„
4.	Stomach, intestines and mesenteric vessels ligatured, and left *in situ*...	„	„	„
5.	Liver only removed...	„	„	„
6.	Liver vessels ligatured; left *in situ*	„	„	„
7.	Organs cut with scissors, and contents of abdominal cavity stirred with a rod	„	„	„
8.	Duodenum and pancreas only removed	„	„	„
9.	Liver and intestines removed, and stomach placed in pelvis	„	over pelvic region	
10.	Stomach and intestines removed, and liver placed in pelvis	„	„ „	„
11.	Stomach and intestines removed, and pancreas and duodenum placed in pocket under skin of right shoulder	„	over inserted pancreas	

These experiments seem to indicate that the removal of one of the larger abdominal organs has little effect on the place or time of the appearance of the fluid, but the complete removal of the liver, stomach and intestines and the replacement of one of these organs in an abnormal situation causes the patch to appear first in that situation.

We suggest that the phenomenon is due to the action of the ferments present in the liver, stomach, intestines and pancreas and that in an

intact body the place at which the action on the abdominal wall is first produced is due in guinea-pigs to the anatomical disposition of the omentum, which partially protects certain portions of the abdominal wall, and partially guides the autolytic fluids exuding from the organs mentioned so that they mix and exert their greatest action on the unprotected area of abdominal wall lying against the cardiac end of the stomach. The action of the ferments allows the fluid to pass through the muscle layers, and so acts on the skin that the hairs become loosened in their follicles, and together with the superficial epithelium are detached from the underlying skin by the exuding fluid.

In order to determine whether the action on the skin was due to moisture rather than to ferment action two further experiments were undertaken. The body of a freshly killed guinea-pig was taken and a testis was inserted under the skin of the right side of the neck, a kidney in the middle line of the back and a piece of small intestine, ligatured at the ends, under the skin of the right thigh. In the same situations in the body of another guinea-pig 1 c.c. of colon contents, small intestine contents and stomach contents were inserted. In the former body the hair began to come off first over the piece of small intestine, a day before the usual patch on the side was noticed. No unusual softening of the skin occurred over the inserted organs. In the second body the skin began to soften first over the spot covering the contents of the small intestine, and on the same day the usual patch was noticed on the left side. No unusual softening of the skin occurred over the areas covering the contents of the stomach or colon.

These experiments seem to show that the contents of the small intestine, presumably due to the enzymes in them, exert a specific action. Other experiments having a bearing on this subject are quoted later.

The Effects of Injection of Creosote Oil into the Blood Vessels.

We have already shown (p. 132) that injections of small quantities of antiseptics into the blood vessels, without treatment of the skin, have little effect in stopping putrefaction, when the atmosphere is moist. The following experiments were carried out in order to ascertain the effects of injection through the carotid artery of varying quantities of creosote oil, when the carcases were kept in a dry atmosphere at a temperature of 60° F.

TABLE XXI.

Showing the effects of the injection of creosote oil into the blood vessels.

	Weight of animal grms	Quantity injected c.c.	c.c. injected per 100 grms	Other treatment	Remarks
A	383	13·6	3·54	None	5th day usual patch. No gas
B	426	10·0	2·36	..	.. ..
C	491	5·8	1·18	..	.. .. .. Distended
D	468	5·5	1·18	Tube in peritoneum	.. ..
E	530	6·3	1·18	Abdomen opened	.. .. .. Little gas
F	508	6·0	1·18	Tube in coecum	.. .. .. Slightly distended
G	473	5·6	1·18	Skin over abdomen treated with 7 c.c. creosote oil	.. ..
H	458	None	None	Skin treated with 23 c.c. creosote oil	.. ..
I	340	..	..	Skin treated with 25 c.c. olive oil	4th day Distended
J	—	..	..	None	5th day skin dissolved over stomach

In all cases the skin became moist and fluid appeared in the usual spot on the left side, and in all gas developed except in the first two, which received the largest injections of creosote oil.

On the 20th day the carcases of these animals were examined for the presence of bacteria in the following way. As much as possible of the hair was removed, and the remainder singed off. The body was then fastened on a board, and the under surface wetted with lysol solution to prevent particles from flying about. Then the ventral surface was seared with a cautery and the abdominal and thoracic walls opened with sterile instruments. Next portions of the liver, lung and thigh muscles were removed with sterile instruments, ground between sterile ground glass plates, and cultures made on agar and in broth. Portions of the contents of the stomach and large intestine were also cultivated.

TABLE XXII.

Showing the results of cultures from the organs of creosote oil injected bodies.

	Lungs	Liver	Muscle	Stomach		Coecum	
A	0	0	0	spore bearing bacilli		spore-bearing bacilli	
B	0	0	colon-like bacilli	..		..	..
C	few colon like	0	..	..		..	+ colon-like
E	0	0	..	..		..	..
G	0	0	..	..		..	
H	colon like	colon-like	..	..	+ colon like	..	+ colon-like
I	..	..	..	..	..	..	..
J	spore bearers	.. + spore bearers	.. + spore bearers	..		..	..

These results show that, according to the method adopted, organisms could not be demonstrated by culture methods in the livers of the injected carcases 20 days after treatment, and that in most cases they could not be demonstrated in the lungs. On the other hand only the largest injections prevented the penetration of intestinal organisms into the muscles. Organisms of the intestinal type were plentiful in the organs when the skin only was treated. It is worthy of note that spore-bearing bacilli were found only in the organs and muscles of the untreated control, *J*. From the stomach contents of all spore-bearing bacilli were cultivated in large numbers, and from the coecal contents both spore-bearing bacilli and colon-like organisms.

Experiment *A* indicates that fluid exudes from a carcase in which the organs and muscles are sterile.

On dissection the condition of the body treated with olive oil was distinctly better than that of the control, showing that treatment of the skin with an oil possessing little, or no, antiseptic properties has some effect in retarding putrefaction under the conditions of this experiment.

Injections of Certain Constituents of Creosote Oil into the Blood Vessels.

A further series of experiments were carried out under the conditions mentioned in the last section, a dry atmosphere and a temperature of 80° F., in order to compare the results of injection of certain of the constituents of creosote oil with the oil itself.

Without the aid of chemical analyses it is difficult to arrange the results of this series of experiments in their exact order. It is clearly evident, however, that each group of substances separated from creosote oil exerts a very great influence in checking putrefactive changes under the conditions of this experiment. The best results were obtained by the injection of phenolic mixtures of 100 % strength, which not only preserved the bodies to an extraordinary degree but inhibited the formation of gas. Bearing in mind our remarks on the stenches from decaying bodies it is interesting to note that a slight rancid smell was noticed when the carcase marked (*d*) was dissected.

The organs of specimens (*e*) and (*f*) were examined for the presence of bacteria by the method described in the last section. The cultures from the lungs, liver and muscle of (*e*) remained sterile, but those from the liver and muscle of (*f*) contained colon-like organisms. In both cases organisms were present in the contents of the stomach and coecum.

TABLE XXIII.

Showing the results of injections of certain constituents of creosote oil into the blood vessels.

	Weight of body	Material injected	Quantity injected	Distension	Fluid exuded, L. side	Dissection	Date of dissection
	grms		c.c.	day	day		day
a	605	creosote oil	7·0	7	9	good preservation	14
b	493	fraction to 317° C.[1]	5·9	6	8	.. ..	23
c	433	.. ..	2·4	6	6	.. ..	23
d	575	phenolic mixt. (100 %)[2]	7·0	never	14	very good preservation	23
e	505	phenolic mixt. (100 %)[3]	6·3	..	5	(see p. 165)	
f	299	.. .. ..[3]	1·2	..	5	.. .	
g	464	"water sol." bases (100 %)[4]	5·9	13	13	good preservation	27
h	485	hydrocarbons and bases[5]	5·9	5	6	slightly decomposed	23
i	780	hydrocarbons[6]	7·1	7	9	good preservation	23
j	477	"residue"[7]	5·9	5	8	slightly decomposed	23
k	451	Control	0	7	9	Organs represented by a dark brown deposit. Smell very bad	

[1] Amounted to 81 % of the creosote oil, and contained 17·1 % of phenolic bodies.

[2] Separated from the fraction of creosote oil distilling between 170—220° C.

[3] Complete mixture of phenolic bodies separated from fraction of creosote oil distilling up to 317° C.

[4] See p. 159.

[5] Remainder of fraction 317° C. of creosote oil after taking out the phenolic bodies, consisting of hydrocarbons and bases.

[6] Hydrocarbons only from fraction 170—220° C.

[7] Residue from distillation of creosote oil up to 317° C., amounting to 19 % of the original oil.

In all cases including (*e*) fluid exuded from the body confirming our hypothesis that the phenomenon is independent of bacterial activity. Smears from the exuded fluids showed numerous organisms in specimens (*b*), (*c*), (*h*), (*i*) and (*j*), few in (*a*) and (*g*) and very few in (*d*) and (*e*).

Injections of Various Reagents into the Blood Vessels.

Having shown that even under the optimum conditions for putrefaction, a moist, almost oxygen free atmosphere and a temperature of 26·5° C., treatment of the skin with creosote oil inhibits putrefaction to a very great extent, the opportunity was offered of ascertaining under these conditions the effects on the tissues of injections into the blood vessels of reagents possessing very different properties. We believe the fluid which exudes results from cytolysis and enzyme activity,

and as it has been asserted that the actions of enzymes are stopped by acids and alkalis exceeding 0·1 (N) our first injections were calculated to produce this strength in the blood. Since the exudation of fluid was not influenced by this procedure we injected in some experiments sufficient to produce a strength of 0·1 (N) in the whole of the water in the carcase, calculating the water as 65 % of the total weight. The bodies of freshly killed guinea-pigs were used. In each case the skin and natural orifices were treated with creosote oil and the reagent was then injected through the carotid artery. The experiments were conducted in the apparatus previously described (p. 127), but the gas produced was not collected. The quantity of fluid which exuded was noted daily, and finally the carcases were carefully dissected, some at the end of five days, and others after 10 and 16 days.

TABLE XXIV.

Showing the results of the injections into the blood vessels of various reagents in carcases with the skin treated with creosote oil.

	Weight of body grms.	Skin treatment, c.c. of creosote oil per 100 grms.	c.c. per 100 grms. injected	Reagent		Fluid exuded per 100 grms.	Date of dissection day	Remarks
A	471	4·66	1·22	(N) Hydrochloric acid	3·65 %	16·3	5	moderate preservation
B	284	3·52	2·11	N/2 Sulphuric acid	2·45 %	19·7	12	very decomposed
C	410	3·54	2·13	1·7 (N) Chromic acid	10·0 %	18·0	12	moderate preservation
D	705*	4·68	1·22	N/10 Arsenic acid	0·71 %	7·1	5	very good preservation
E	371*	3·50	1·31	N/5 ,, ,,	1·42 %	12·4	16	poor preservation
F	312*	3·52	2·6	N/5 ,, ,,	1·42 %	7·7	16	good preservation
G	396*	3·50	2·6	N/5 Arsenious acid	0·96 %	7·1	16	very good preservation
H	417*	4·6	1·22	(N) Orthophosphoric acid	4·9 %	11·5	5	moderate preservation
I	479*	3·55	2·44	2·5 (N) ,, ,,	12·25 %	23·4	16	,, ,,
J	556	4·67	1·22	(N) Monopotassium phosphate	13·6 %	24·4	5	,, ,,
K	444	4·66	1·23	(N) Formic acid	4·6 %	18·2	5	complete maceration
L	355	3·50	1·23	4 (N) ,, ,,	18·4 %	25·3	16	,, ,,
M	483	3·54	1·9	4 (N) ,, ,,	18·0 %	23·3	12	well preserved
N	555	4·66	1·22	(N) Lactic acid	9·0 %	17·3	5	very decomposed
O	499	4·60	1·21	(N) Soda	4·0 %	16·6	5	moderate preservation
P	387	3·50	1·22	3 (N) Ammonia	5·1 %	25·8	10	complete maceration
Q	368	3·53	1·87	10·6 (N) Ammonia	18·0 %	23·3	11	moderate preservation
R	490	4·63	1·22	2·5 (N) Sodium fluoride	10·5 %	18·7	5	,, ,,
S	370	3·50	1·22	(N) Sodium nitrite	6·9 %	11·9	10	very good preservation
T	373	3·50	0	Liver painted with strong mercuric chloride		24·1	16	poorly preserved

* Orthophosphoric, arsenic and arsenious acids were considered as dibasic in making up the solutions, and their molecular weights taken as representing two equivalents.

Dissections of bodies used in this experiment.

The bodies were supported on glass pedestals head downwards resting on the shoulder.

A. Injected with hydrochloric acid. Dissected 5th day. 77 c.c. of brownish fluid had exuded. No smell. *Liver* gas bubbles throughout. *Stomach* soft, full of gas. *Kidney* soft. *Spleen* very soft. *Abdominal wall* on left side dark and emphysematous. *Muscles* pink and slightly soft. *Remarks.* A little smell of rancid type. The quantity of acid injected was evidently insufficient to prevent marked activity of the gas-forming organisms, nor did it seem to have interfered with autolysis or the exudation of fluid.

B. Injected with sulphuric acid. Dissected on the 12th day. 56 c.c. of fluid had exuded. *Remarks.* None of the internal organs or muscles distinguishable. Remains alkaline to litmus.

C. Injected with chromic acid. Dissected 12th day. 74 c.c. of fluid had exuded. Tissues everywhere dark green in colour, probably through the reduction of the chromic acid. *Liver* not soft, full of gas bubbles. *Kidney* not soft. Cardiac end of *stomach* wall dissolved. *Muscles* moderately good. *Smell* very little, if any. *Remarks.* The carcase was moderately well preserved. Alkaline to litmus. Neither gas formation nor exudation of fluid inhibited.

D. Injected with arsenic acid. Dissected 5th day. 50 c.c. of red fluid had exuded. *Lungs* red, oedematous but well preserved. *Liver* pale, normal in size; no gas bubbles; sinks in water; oedematous. *Kidney* looks normal, but soft and oedematous. *Spleen* soft. *Stomach* wall very soft. *Abdominal wall* normal in appearance and consistency. *Muscles* excellent in appearance, but oedematous. *Remarks.* The fluid exuded very slowly, and the total amount was small, but all the organs were oedematous. The carcase was very well preserved.

E. Injected with arsenic acid. Dissected on the 16th day. 46 c.c. of clear, red fluid, containing some sediment, exuded. *Lung* very oedematous, and fluid in thorax. *Liver* much disintegrated, numerous bubbles. *Muscles* soft and disintegrated. *Subcutaneous tissues* oedematous, especially in the dependent parts. *Remarks.* This carcase was not nearly so well preserved as *F*, *G* or *S*.

F. Injected with arsenic acid. Dissected on the 16th day. 24 c.c. of clear, red fluid, containing some sediment. *Thorax* some red fluid. *Lungs* oedematous, but normal looking. *Liver* gas bubbles. *Muscles* of hind leg dry and pale. Collections of gas in the retroperitoneal tissues. *Remarks.* This carcase was not so well preserved as *G*.

G. Injected with arsenious acid. Dissected 16th day. 28 c.c. of reddish-brown fluid exuded very slowly. No smell. *Hair* loose over the usual patch and the abdomen, but not elsewhere. *Thoracic organs* normal in appearance, but oedematous; soft clot in heart. *Liver* yellow, normal in size and consistency; no gas bubbles; sinks in water. *Kidney* normal in appearance. *Intestines* very well preserved, and almost normal in consistency. Mesentery oedematous, and abdominal walls stained black over the intestines. *Muscles* of hind legs and psoas exceedingly well preserved; not oedematous; muscles of dependent parts oedematous. No

collections of gas in the carcase, and at no time was distension observed. *Remarks.* This carcase was very excellently preserved.

Note. *F* and *G* were treated with the same amount of arsenic the former in the form of arsenic and the latter in the form of a solution of arsenious oxide.

H. Injected with orthophosphoric acid. Dissected on the 5th day. 48 c.c. of red-brown fluid exuded. *Thoracic organs* normal in appearance and consistency. *Liver* pale, soft, normal in size; numerous bubbles. *Kidney* very dark, soft, but normal in size. *Spleen* dark and soft. *Stomach walls* disintegrated where in contact with liver. *Abdominal walls*, except at usual left patch, normal in appearance. *Muscles* almost normal in appearance and consistency; psoas very soft and oedematous. Subcutaneous emphysema. *Remarks.* Moderately well preserved. Gas present throughout the tissues.

I. Injected with orthophosphoric acid. Dissected on the 16th day. 112 c.c. of red-brown fluid had exuded. *Liver* full of gas bubbles. *Stomach* tough, and *intestines* well preserved. *Muscles* pink, slightly soft, but not much decomposed. Gas in all the tissues. *Remarks.* Moderately well preserved.

J. Injected with monopotassium phosphate. Dissected on the 5th day. 136 c.c. of chocolate coloured fluid had exuded. *Thoracic organs* very soft. *Liver* pale, soft, full of gas bubbles. *Kidney* soft. *Spleen* soft. *Stomach wall* emphysematous. *Intestines* contain much gas. *Muscles* normal in appearance and consistency. *Abdominal wall* very soft. *Remarks.* Moderately well preserved.

K. Injected with formic acid. Dissected on the 5th day. 80 c.c. opaque, dirty-looking fluid had exuded. *Lungs* and diaphragm disintegrated. *Liver* and *kidney* very much disintegrated. *Stomach walls* dissolved. *Muscles* diffluent, and macerated from the bones. No gas collections found anywhere. Probably decomposition has advanced so far that the gas has been set free. *Remarks.* The carcase is very much macerated.

L. Injected with formic acid. Dissected on the 16th day. 90 c.c. of red-brown turbid fluid with much sediment. *Remarks.* The carcase drops to pieces. No organ was recognizable, and the muscles have been macerated from the bones.

M. Injected with formic acid. Dissected on the 12th day. 114 c.c. of fluid had exuded. *Liver* dark, shrunken, soft but no gas bubbles seen. *Kidney* very soft. *Stomach wall* tough. *Intestines* tough and full of gas. *Muscles* soft but look normal. Collections of gas in the retroperitoneal and other connective tissues. *Remarks.* The carcase was acid to litmus and well preserved.

Note. Reckoning about 65 % of the carcase as water sufficient formic acid had been injected to make the percentage of acid in the water of the body 0·35 % in the case of *L* and 0·5 % in the case of *M*. The former was much macerated and the latter well preserved. (See *P* and *Q*.)

N. Injected with lactic acid. Dissected on the 5th day. 96 c.c. of red fluid had exuded. *Remarks.* The carcase was similar in all respects to *K*.

O. Injected with soda. Dissected on the 5th day. 83 c.c. of red-brown, opaque, dirty-looking fluid exuded. *Liver* brown, full of bubbles, and collapsed. *Kidney* soft and dark. *Spleen* dark and very soft. *Stomach walls* disintegrated where in contact with liver. *Muscles* pink and soft. Collections of gas present. *Remarks.* Condition similar to *A*.

P. Injected with ammonia. Dissected on the 10th day. 100 c.c. of red-brown, turbid fluid with much sediment had exuded. None of the organs was recognizable, and the muscles had been macerated from the bones. *Remarks.* This carcase was the most macerated of the series.

Q. Injected with ammonia. Dissected on the 11th day. 82 c.c. of fluid had exuded. *Skin* over abdomen very thin. *Liver* very soft, very numerous gas bubbles. *Kidney* very soft. *Stomach* anterior wall disintegrated. *Intestines* moderately well preserved. *Muscles* well defined but soft, and easily detached from the bones. Little smell, apparently from stomach contents. All tissues alkaline to litmus. Collections of gas. *Remarks.* The body was moderately well preserved.

Note. In *P* the ammonia in the water of the tissues was 0·08 %, in *Q* 0·5 %. The former was macerated, the latter moderately well preserved.

R. Injected with sodium fluoride. Dissected on the 5th day. 92 c.c. of red-brown fluid had exuded. *Thoracic organs* well preserved. *Liver* soft, collapsed, full of gas bubbles. *Kidney* not very soft. *Spleen* soft. *Stomach wall* much disintegrated. *Muscles* very well preserved, but slightly soft. Psoas well preserved. *Remarks.* The carcase was moderately well preserved.

S. Injected with sodium nitrite. Dissected on the 10th day. 44 c.c. of clear, light yellow fluid had exuded, with some whitish sediment. Faint fresh blood odour. *Lungs* and *heart* very well preserved. *Liver* normal in size and appearance, no gas bubbles seen; smallest fragments sink in water. *Kidney* soft, but structure visible. *Intestines* slightly soft. No collections of gas or subcutaneous emphysema. *Muscles* very firm, and difficult to tear, remarkably well preserved, and not oedematous; psoas soft. *Hair* comes off easily over usual patch and abdomen, but not elsewhere. *Remarks.* This is probably the best preserved carcase of the series, the organs and muscles being in remarkably good condition. The fluid and organs were free from discoloration from blood pigment. The fluid exuded very slowly; no gas had been produced.

T. Liver treated with strong mercuric chloride. Dissected on the 16th day. 90 c.c. of brown fluid, with some smell, exuded. *Liver* very soft but shows no gas bubbles, and sinks in water. *Thoracic organs* very soft, and fluid in cavity. *Intestines* soft. *Muscles* diffluent and macerated from the bones. *Remarks.* The carcase is decomposed, but not so badly as *P.* The treatment of the liver seems to have preserved it to some extent but has not influenced general putrefactive changes.

The conditions found on post mortem examination of the bodies *F*, *G* and *S* were so remarkable that we analysed filtered samples taken from the whole of the fluid which had exuded. 5 c.c. portions were taken, 22 c.c. of water added and made up to 250 c.c. with 97 % alcohol. The results are given in Table XXV.

As we believe that no bacterial putrefaction occurred in these carcases, we propose to adopt a ratio of 0·45 : 1 as a standard for comparing the extent of putrefaction in carcases after a few days' exposure. It is of interest to compare these ratios with that obtained in the left gluteus of the white horse after eight and a half months' exposure. In

TABLE XXV.

Showing the results of the analyses of fluids which had drained from the bodies of guinea-pigs injected with arsenic acid (F), arsenious acid (G) and sodium nitrite (S).

	Fluid collected	Quantity taken	In two-fifths of filtrate: Volatile bases c.c. N/10 acid neutralised	In two-fifths of filtrate: Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
F	24 c.c.	5 c.c.	2·00	4·65	0·43 : 1
G	28 ,,	5 ,,	1·80	4·40	0·41 : 1
S	44 ,,	5 ,,	2·50*	5·50	0·45 : 1

* A very careful inquiry, involving several different tests, was made to determine the presence of nitrite in the fluid, but all including the extremely sensitive metaphenylene diamine reaction gave negative results, showing that no traces of the nitrite remained. We suggest that the nitrite had been reduced to ammonia. If this is the case we calculate that the volatile base figure should be reduced by 0·4 c.c. to allow for the ammonia from this source. It is of interest to note that if this correction is made *G* and *S* give almost identical ratios. As no nitrite was present in the fluid there was no necessity to adopt van Slyke's method for the amino-nitrogen instead of the formyl titration method.

discussing this subject (p. 207) we attempt to explain that the ratio of about 1·5 : 1 found in fresh muscle should fall, if uncomplicated by the action of putrefactive bacteria, until the proteolytic ferments cease to act.

In four cases we collected the exuded fluid on the 4th or 5th days, and on the 11th or 12th days, and analysed samples from the portions taken at these times.

TABLE XXVI.

Showing the results of analyses at different times of the fluids draining from the bodies of guinea-pigs injected with formic acid (M), chromic acid (C), sulphuric acid (B) and ammonia (Q).

	Date of collection	Total collected	Quantity taken	In two-fifths of filtrate: Volatile bases c.c. N/10 acid neutralised	In two-fifths of filtrate: Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
M	1—5 days	79 c.c.	5 c.c.	2·20	5·45	0·40 : 1
	5—12 ,,	35 ,,	5 ,,	2·95	6·40	0·46 : 1
C	1—4 ,,	46 ,,	5 ,,	1·40	2·60	0·54 : 1
	4—11 ,,	28 ,,	5 ,,	5·00	5·00	1·0 : 1
B	1—4 ,,	28 ,,	5 ,,	3·25	5·75	0·57 : 1
	4—11 ,,	28 ,,	5 ,,	13·35	3·50	3·82 : 1
Q	1—5 ,,	50 ,,	5 ,,	5·70	2·65	—
	5—12 ,,	36 ,,	5 ,,	14·80	3·55	—

All the tissues of the carcase injected with formic acid (*M*) were acid to litmus. The two samples of fluid gave similar results on analysis, indicating no bacterial decomposition.

The tissues of the body injected with chromic acid (*C*) were alkaline to litmus at the time of dissection. The ratios in the first and second samples appear to indicate that some slight bacterial action was in progress. The figures in column 5 seem to show that enzyme action was partly inhibited at any rate during the first four days.

At the time of dissection the tissues of the body injected with sulphuric acid (*B*) were strongly alkaline to litmus. Bacterial action was apparently slight during the first period, but considerable during the second period. The injection of the dilute acid corresponding to 0·08 % in the water of the whole body (see *M*) has perhaps contributed largely to the maceration, owing to its cytolytic effects.

Sufficient ammonia was injected in *Q* to make a concentration of 0·5 % in the whole water of the body, assuming it became evenly distributed. The injected ammonia contained in the 2 c.c. of fluid used for analysis should neutralise 5·9 c.c. N/10 acid. After making some such allowance for the injected ammonia it is evident that bacterial change has occurred, at any rate in the second sample of fluid.

Remarks. (*a*) The substances injected do not prevent the action of enzymes, when in their natural environment in the tissues. (*b*) The reagents seem to have been fixed in the tissues, since tests for arsenic, nitrites, and sulphuric and chromic acids in the fluids were negative. Possibly traces of formic acid were present. (*c*) The presence of small quantities of creosote oil in the fluids collected in the bottles appeared to prevent further bacterial change after exudation. (*d*) It would be of interest to repeat these experiments omitting the skin treatment with creosote oil.

In regard to these experiments the following points are noteworthy:

(1) Treatment of the skin alone with creosote oil inhibits putrefaction to a great extent (see p. 132).

(2) The injection of weak solutions of certain reagents hastens putrefaction in spite of the treatment of the skin. These reagents exhibit well-marked haemolytic properties in test tube experiments, and probably assist putrefaction by causing disintegration of the cells with which they come into contact.

(3) Bodies injected with weak formic acid (*K*) and weak lactic acid (*N*) became completely disintegrated within 5 days. The increase of formic acid up to 0·07 (N) in the blood is not advantageous, but if the amount is increased to 0·1 (N) in the whole water of the carcase the body is well preserved, though gas is produced and fluid exuded.

(4) Both arsenic preparations showed remarkable preserving properties, though in very weak strength.

(5) The attempt to differentiate between the effects of the two potent replaceable hydrogen atoms of the orthophosphoric acid gave negative results (see *H* and *J*).

(6) The injection of ammonia so as to produce a strength of 0·3 (N) in the blood or 0·046 (N) in the whole water of the carcase resulted in advanced disintegration in a few days, though when the strength reached 1·6 (N) in the blood or 0·25 (N) in the water of the carcase the body was moderately preserved. The macerating action of the weak ammonia is perhaps a factor in disintegration. We have ascertained from other experiments that some organisms can live in the latter strength of ammonia. The ammonia and other 'volatile bases' produced by putrefactive organisms would exercise a disintegrating effect, especially when sufficiently diluted with water. This is probably one of the contributing causes of the more rapid disintegration of carcases when exposed to rain.

(7) The most remarkable result was obtained by the injection of sodium nitrite. The absence of pigmentation of the fluid and tissues was noteworthy.

The following reasons induced us to experiment with sodium nitrite. Many enzymes are regarded as protein in nature and may possess free amino groups in their molecular structure. If this hypothesis is correct the nitrous acid formed by the interaction of the nitrite with the acids developed in the tissues after death might prevent the action of these enzymes by attacking these amino groups.

(8) Gas was produced in all cases except *G* and *S*.

(9) Fluid exuded from every carcase. The amount of fluid which exudes from a body should be considered in relation to the condition of the tissues on dissection. Autolytic ferments tend to macerate the tissues, and the extent to which maceration goes on determines the amount of fluid which exudes, and the appearance of the tissues on dissection. Agents with considerable cytolytic powers contribute to the maceration. In the carcases injected with weak ammonia and weak formic and sulphuric acids so much maceration had occurred that the muscles were detached from the bones. Weak acids and bases seem to facilitate cytolysis to a great extent. The action of arsenic must be considered in regard to its specific effect. Sodium nitrite is a salt. The fluid exuded from the bodies injected with these reagents probably consists of serum, the water of the injected fluid and water from the gut. The

arsenic injected bodies yielded less fluid than the one injected with nitrite, and in this case fluid was present within the muscles. The liquid found in the carcases injected with weak ammonia and weak formic and sulphuric acids had nearly the same appearance as the fluid that exuded, but contained more sediment. The subject requires further investigation.

The Results of injecting Various Reagents into the Blood Vessels of Carcases exposed out-of-doors.

In this series of experiments the blood vessels of freshly killed rabbits were injected through the femoral artery. Consequently the reagent did not pass into the vessels of the leg on the side used for injection and the limb acted to some extent as a control. In most cases the skin and wound were treated with sufficient creosote oil to cover the whole surface, and a small quantity was poured into each of the natural openings. The carcases were exposed, lying on their right sides, on the grass without protection of any kind. They were inspected daily and the conditions found carefully recorded. These experiments were carried out at the end of August when the weather was warm and showery, and numerous flies were ready to lay their eggs on all suitable material.

These experiments, which preceded those recorded in the last section, were designed to test the value of injections of various types of disinfectants into the blood vessels of carcases exposed to varying weather conditions. The experiments also gave much information on the protective action of creosote oil, when applied to the skin, its power to repel flies and prevent the laying of eggs. The effects of the injection of creosote oil with and without treatment of the skin were also compared.

The carcases remained on the ground for nine weeks and were then dissected.

A. No fly eggs or maggots were noticed at any time on the carcase, and the external appearance was very satisfactory throughout. On dissection the face muscles were soft, thoracic organs and liver soft, but normal in colour and shape. Intestines normal in appearance, but a little soft. Subcutaneous tissues slightly disintegrated under the skin of the back. The injected leg was much decomposed. *Remarks.* The carcase was moderately well preserved.

B. No fly eggs or maggots were noticed at any time on the carcase, and the external appearance was very satisfactory throughout. On dissection the tissues resembled those of a freshly killed animal in colour, contour, size and consistency. The serous surfaces and fasciae showed the normal glistening appearance. There

TABLE XXVII.

Showing the methods used in treating the bodies of rabbits exposed out-of-doors.

	Weight of body in lbs.	c.c. of creosote oil per lb. used in skin treatment	c.c. of reagent injected per lb.	Reagent*
A	4·5	21	10	10 % hydrochloric acid
B	3·5	15	14	1 % arsenic acid
C	5·0	20	18	1 % mercuric chloride in 5 % sodium chloride solution
D	4·5	18	21	5 % potassium bichromate
E	6·25	0†	0	100 c.c. of 5 % pot. bichromate + 2 % bile applied to peritoneal surfaces and natural orifices
F	6·25	19	15	10 % liver of sulphur ‡
G	4·75	14	20	5 % phenol
H	4·5	0†	19	creosote oil
I	5·5	15	9	,, ,,
J	4·0	16	0	16·25 c.c. creosote oil applied to peritoneal surfaces

* All the reagents, except creosote oil, were in aqueous solution.

† No skin treatment.

‡ Free sulphur filtered off.

was no evidence of oedema or gas formation. The leg on the injected side was however much decomposed. *Remarks.* Preservation perfect.

C. A few fly eggs were deposited on the 15th day, but no maggots were ever seen on the carcase. The external appearances were satisfactory throughout. On dissection the under surface of the head was decomposed. The thoracic organs were well preserved; liver soft; intestines well preserved, but soft. The hair came off moderately easily over the abdomen. Muscles of back rather soft. The injected leg was much decomposed. *Remarks.* The carcase was not quite so well preserved as *A*.

D. A few fly eggs were found on the 21st day, but no maggots were ever seen on the carcase. The external appearances were satisfactory throughout. On dissection the facial muscles were very soft; the thoracic organs well preserved, but fluid was found in the pleural cavity. Liver was well preserved. Intestines were well preserved with dark patches on their surfaces. No smell. Muscles soft and greenish in colour, probably due to the reduction of the bichromate. The injected leg was much decomposed. *Remarks.* The carcase was moderately well preserved.

E. On the second day numerous flies were seen on the carcase, and large numbers of eggs had been deposited. By the 10th day there were numerous maggots, the hair was coming off and the muscles were disintegrating. By the 14th day the flesh was much decomposed, and the smell was bad. By the 21st day the maggots had eaten the greater part of the carcase.

F. In 14 days a few eggs were found in the mouth and coat, and large maggots in small numbers were found in the thigh muscles on the 28th day. Maggots

had also made a large hole into the abdomen. On dissection the muscles round the mouth were slightly disintegrated. All the organs were very soft, but their contours were preserved; muscles pale and soft, but not disintegrated. The hair came off easily in most situations. The injected leg was much decomposed. *Remarks.* The carcase was comparatively poorly preserved.

G. A few eggs were deposited on the 12th day, but except on the injected leg maggots were never seen. On dissection the face muscles were found well preserved; thoracic organs soft and some fluid in the cavity; liver soft; intestines soft, but moderately preserved. Muscles well preserved, but soft. Injected leg muscles soft, but not very much disintegrated. The hair came off moderately easily; very little smell. *Remarks.* This carcase was moderately well preserved.

H. Many eggs were deposited by the 2nd day. These had not hatched on the 5th day. On the 9th day a few small maggots in the coat. 13th day many small maggots found dead. On the 19th day the muscles of the injected leg had been eaten by maggots. On 31st day no maggots were found on the carcase, indicating that they could not eat the portions of the body reached by the injection. Dissection; hair came off very easily everywhere, and the skin was disintegrated over the abdomen, and slightly over the thorax; face muscles very well preserved and firm; thoracic organs firm and normal in shape; intestines wonderfully well preserved with asphalt-like smell; muscles soft but well preserved. *Remarks.* With the exception of the skin and underlying tissues the carcase was well preserved.

I. On the 19th day a few eggs were found on the coat, but no maggots were ever seen on this carcase. Dissection. The skin of one side was removed and the carcase photographed (Pl. V, Fig. 14). The figure shows the wonderful state of preservation of the carcase. In this state it was allowed to remain in a room for a month, and caused no nuisance. During this month the muscles became shrunken and very hard, and the body looked like a dark mummy. On dissecting the surface covered with skin it was found little changed. Though flies were present in the room no eggs or maggots were found on the carcase. *Remarks.* It will be noticed that twice the quantity was injected into *H* and yet *H* was not nearly so well preserved as *I*, which received skin treatment together with injection. The beneficial effects of skin treatment are very evident, when carcases such as these are compared side by side. The access of water and air has permitted the organisms present in the skin to produce some disintegration in the peripheral tissues of the injected carcase *H*.

J. This body lay on its back. On the 2nd day rain water had collected in the exposed abdominal cavity. On the 19th day a few eggs were deposited. No maggots were ever found on the carcase, and its appearance was satisfactory throughout. Dissection. The hair came off moderately easily; the facial muscles were well preserved; the exposed coils of intestine hard, but those underneath were normal in shape and consistency; muscles normal in consistency, colour and shape. *Remarks.* This carcase was very well preserved.

In all cases, except *E* and *H*, the skin was very tough, in spite of the fact that the carcases were frequently wet with rain. In no case, except *E*, was there any appreciable smell.

In regard to these experiments the following points are noteworthy:

(1) Even under weather conditions very favourable to putrefaction small carcases exposed in the open can be preserved for months.

(2) Treatment of the skin with creosote oil prevents the external conditions from nullifying any antiseptic properties which the injected fluid may possess.

(3) Treatment of the skin with creosote oil repels flies, and preserves the carcase from the attacks of maggots.

(4) An extraordinarily good result was obtained when a very dilute solution of arsenic acid was used for injection, amounting to a concentration of 0·046 % in the water of the body (see p. 173).

(5) Excellent preservation of the body can be obtained even when the abdominal cavity is opened and the organs exposed, if the peritoneal surfaces and skin are treated with creosote oil.

(6) An open wound was left at the site of injection and none of the injection fluid passed into the vessels of this limb. In every case the muscles of this limb were in an advanced stage of decomposition.

The Distribution of Fluids injected into the Blood Vessels.

In considering the effects of injections of reagents into the blood vessels it is desirable to know to what extent distribution occurs when fluids are introduced soon after death into vessels containing blood. We injected the bodies of guinea-pigs through the carotid artery and the bodies of rabbits through the femoral artery, and never experienced any difficulty in making the fluids pass into the vessels. Very early in the process of injecting the fluid may be seen passing up the arteries and capillaries of the ear, and finally passing down the veins. If a loop of intestine be exposed the same phenomenon may be noticed, and by cutting the skin of the feet it may be shown that the fluid passes into the vessels of the extremities. In the case of fluids insoluble in water, such as creosote oil, we believe some of the blood is pressed to the sides of the vessels, but the greater part accumulates in the large veins. This is best shown by injecting colourless fluids, such as medicinal paraffin. In our endeavour to ascertain with more certainty the distribution of the fluid the vessels of a guinea-pig were injected with milk, and sections prepared of the liver, lung, kidney and muscle. Fat was found in large quantities in the larger and smaller vessels of the lung and kidney, but its distribution in the vessels of the liver and muscle was very irregular.

Substances soluble in the water of the tissues doubtless diffuse out of the vessels, and act upon the cells of the body.

Injections with creosote oil, or the phenolic bodies separated from it at full strength, even in small quantities cause immediate rigor in all the muscles, which remain in this condition for days. The other groups of constituents of creosote oil do not produce this effect.

SUMMARY.

Temperature has a profound influence on the rate at which changes appear in a carcase. A very low temperature indefinitely postpones the apparent changes.

The optimum temperature seems to coincide with that which is most suitable to the activities of ferments and bacteria present in the living body. Alterations in temperature, such as occur in exposed carcases, during the day and night, probably influence putrefaction by their effects on bacterial activity, antagonism, and symbiosis. Variations in temperature conditions may produce changes more profound than would appear likely on superficial consideration. The optimum temperature for one group of organisms is different from the optimum conditions for another. Changes in temperature, therefore, especially if they are of some duration, are apt to favour some groups at the expense of others, and influence in this way the whole sequence of subsequent events.

Water is necessary to the growth of bacteria. It permits of their migration in the tissues, allows their ferments to act, distributes by diffusion the substances on which they live, and dilutes the toxic products of their activity.

The effects of temperature and moisture cannot be dissociated for it is in combination that these factors exert their greatest influence.

In a hot dry atmosphere the removal of water by evaporation may be so rapid that a small carcase may become desiccated before putrefaction has advanced far. In hot arid countries a variety of influences inimical to putrefaction are at work, especially when carcases are resting on dry sand. The strong sunlight tends to destroy the skin organisms, which we believe to be the most important agents in producing putrefaction. The skin soon becomes horny in consistency, imprisoning the organisms within it, and preventing their invasion of the tissues. The horny skin protects the carcases from moisture from any source, and the exudation of fluid finally results in mummification.

In a hot moist atmosphere sunlight is robbed of much of its bactericidal power, and the conditions most favourable to putrefaction prevail. Moisture from the air, rain water and water from the ground are absorbed and keep the skin moist. Suitable conditions for the growth and penetration of skin and soil organisms are thus created. Very soon the superficial layers of the epidermis are loosened and the sodden skin permits the autolytic enzymes, passing from the deeper parts, to exert their action on it and assist the putrefactive organisms. The aerobic conditions now prevailing in the skin aid disintegration. The protective influence of the skin is thus lost, and water has free access to the underlying tissues, where it exerts its elutriating and solvent effects. The products of putrefaction are diluted and removed and the dissemination of organisms favoured. Oxidation now plays some part in the process and disintegration proceeds rapidly. The presence of maggots seems to hasten putrefaction.

Such considerations ought to be sufficient to suggest the inadvisability of treating decomposing animal matter with watery solutions of antiseptics as is so often done. Here we may point out that the flesh can be completely macerated from the bones in six days, if a carcase is kept in water at 37° F. The progress is especially rapid, if the water is changed after three days, and the products which seem to inhibit bacterial growth removed.

Within a few days of death, the time varying with the conditions, certain phenomena are noticed if a carcase is kept under observation. It becomes distended with gas, fluid exudes from it, and the skin over certain areas becomes green. These phenomena are usually regarded as evidences of putrefaction. Since in properly treated carcases they are not followed by disintegration of the proteins, which form the main constituents of the body, and since they appear to be due partly to enzyme action and partly to the action of intestinal bacteria of the colon-type on such constituents as carbohydrates, and not to the action of putrefactive organisms, we consider that we are justified in regarding these phenomena as precursors of true putrefactive changes. By the latter term we understand the disintegration of the tissues by putrefactive organisms, a process accompanied by the elimination of foul-smelling products.

The evidence on which this conclusion is based has been given in detail in this section, and here we propose to discuss only the more important considerations.

The evolution of gas.

Under suitable conditions a carcase soon becomes distended with gas. This is due in the first place to the production of gas in the intestines from the carbohydrates of the food, and secondly to the production of gas in the liver and other tissues mainly from the dextrose, which is present before death or is produced after death by ferment action from glycogen, glucoproteins, etc., and hydrogen sulphide from free cystine. At 26·5° C. the production of gas from the tissues reaches its maximum about the third or fourth day. We believe that this early gas formation is due principally to the action of intestinal organisms, which are known to pass into the organs after death.

This view is supported by the results of experiments on carcases treated in various ways, which remained excellently preserved in spite of a large initial formation of gas. In one experiment (p. 138) on an opened carcase as much as 4·5 c.c. of gas per grm. more than would be sufficient to distend the carcase was produced, and subsequently every part of the body was found to be well preserved. We therefore contend that though the carcase becomes unsightly early distension is no criterion of true putrefaction.

Subsequent gas evolution, especially large daily productions in the second and third weeks, is some guide to the extent of putrefactive changes. The later gas arises from the disintegration of proteins, and the decarboxylation of the resulting amino acids, etc. A comparatively large total gas production per unit weight is associated with considerable bacterial disintegration.

Gas production in the tissues may be prevented by the injection of suitable reagents into the blood vessels.

The exudation of fluid from carcases.

Fluid, which is at first clear and yellow, begins to drain from the body after a few days. In small carcases it first appears, separating the superficial epidermis from the deeper layers of the skin, on the left side in the stomach region. We have shown that fluid was always exuded even from excellently preserved carcases, which produced no gas, the organs of which appeared to be sterile. The exudation of fluid is therefore not an indication of bacterial action or of putrefaction. We think that this fluid consists of serum, water from the cells and liquid from the intestines, and escapes owing to the action on the cells of intestinal and autolytic enzymes. Fluid also exudes from sterile

organs excised from the body and kept in sterile bottles. In untreated carcases fluid drains from them continually and becomes opaque, red-brown and offensive.

Chemical analyses of putrefying substances.

The study of the actions of pure and mixed cultures on sterilised materials, while affording evidence of great value on the biology of certain species of bacteria, and the effects of symbiosis, omits to take into consideration factors which operate in the very complicated processes of putrefaction as it occurs in nature. Natural putrefaction is a process which results from the combined actions of enzymes and various groups of bacteria. By applying suitable methods to the products figures should be obtained, which indicate the collective results.

We have studied these processes as they occur in (1) finely divided animal material in suspension and (2) small carcases under various conditions. We submit a method of determining the ratios of volatile bases to amino acids in the products, which we claim shows the collective results at any stage. The application of this method to the tissues of treated and untreated carcases and the fluids exuding from them reveals the comparative power of disinfectants. Descriptive methods only enable us to discriminate between antiseptics of very different power, but this method enables us to place disinfectants of moderate power in order of merit.

By applying this method to cultures in tryptic digests and amino acid mixtures it should be possible to compare the effects of various species of organisms in pure cultures and in combination.

Stench and deodorants.

The stench from a decaying carcase is a combination of odours and varies in character at different times. The component odours of the stench mainly arise from organic bases and organic acids and sulphur compounds.

Deodorants of a purely acid nature can only fix the bases, while setting free the organic acids responsible for rancid odours. In like manner basic deodorants fix organic acids and set free bases. On the other hand some deodorants, such as oxidising agents, may destroy substances giving rise to odours and not merely fix them. Chemical action resulting in such destruction may be facilitated by the deodorant containing solvents for the constituents of the odour. Some deodorants

only dissolve noxious substances and hold them in solution for longer or shorter periods, according to their rate of evaporation. The period during which a deodorant remains operative depends to a large extent on its rate of evaporation, degree of solubility in water and its power of stopping putrefactive changes in the substances with which it comes into contact.

In estimating the actions of a deodorant it must be remembered that some reagents used for this purpose affect the nasal mucous membrane.

We consider that a perfect deodorant should contain chemical substances capable of eliminating all the constituents, which go to make up the stench. We are only acquainted with one fluid, with a not unpleasant smell, creosote oil, which possesses the necessary constituents and characters of a cheap and satisfactory deodorant.

The skin in relation to putrefaction.

Experimentally the conditions assisting putrefactive changes are exceptionally favourable when the bodies of small animals are kept in bottles at a temperature of 26·5° C. Even under these conditions the carcases can be excellently preserved if the skin is treated thoroughly with such fluids as creosote oil. The application of such fluids acts in three ways, (1) by killing many of the skin organisms, (2) by hardening the skin and thus increasing its protective properties and imprisoning organisms in it, and (3) by preventing the entrance of water and air into the carcase. Treatment of the skin does not interfere with the invasion of the tissues by intestinal gas-forming organisms, and yet in skin treated bodies disintegration of the tissues does not occur. We therefore believe the skin to be the chief source of putrefactive organisms. Chemical analyses of the tissues of skin treated bodies and the fluids draining from them confirm the opinion formed on dissections of the bodies.

These carcases though excellently preserved become distended with gas and exude fluid. In the hope of finding some means of inhibiting the activity of the intestinal bacteria, which cause the production of gas, and of checking the production of fluid through cytolysis and enzyme action, we injected the vessels of skin treated animals with various reagents.

Injections into the blood vessels.

In regard to the effects of injections into the blood vessels, uncombined with skin treatment, we suggest the following points for consideration.

The injection of a disinfectant produces an effect depending upon its nature, and the amount employed. The amount required to prevent putrefaction is influenced by the degree of fixation of the reagent by the tissue constituents. Aqueous solutions are diluted by the fluids of the body, and fixation commences at once. Oily fluids when injected are distributed throughout the smallest capillaries and diffusion of the bactericidal constituents contained in them is very slow. The injection of sufficient quantities of antiseptics in this manner is advantageous because (1) being protected in the oily particles they are not immediately fixed; (2) they continue to act for long periods, and (3) they are carried to and remain in those peripheral parts of the body it is most desirable to reach. The effects on organisms depend on (1) the concentration of free disinfectant in their vicinity, and (2) the length of time during which it acts. We have found the organs and muscles sterile after injection with phenolic bodies (p. 164). A large injection of creosote oil results in a fair degree of preservation of a carcase.

While preservation by means of injection alone requires the use of large quantities of disinfectants, extraordinary good results can be obtained by combining injection with skin treatment. For example bodies skin treated with creosote oil and injected with arsenious oxide or sodium nitrite never produced gas and exuded little fluid and were exceedingly well preserved though kept for long periods under conditions very favourable for putrefaction.

Small quantities of weak acids and ammonia seem to hasten dissolution even when injection has been combined with skin treatment.

The value of a reagent for stopping putrefaction should be gauged according to its capacity to preserve a body kept under conditions favouring rapid putrefaction.

Part III. Experiments on Maggots and Exposed Carcases.

Experiments on Maggots with Coal-Tar Oils and their Constituents.

In summer time fly maggots play such an important part in the destruction of exposed carcases, both by devouring the tissues and by altering the conditions under which putrefaction is proceeding that it may be best at this point to consider the action of coal-tar products on them.

In the series of experiments described in the following pages very large maggots, taken from carcases, were employed, since in preliminary tests we had found them to be much more resistant to the action of chemical agents than young and half grown specimens. In most cases six maggots were treated for a definite time with the reagent, then placed on blotting paper to remove the greater part of the reagent and finally in clean glass vessels in which the results could be watched.

TABLE XXVIII.

The effects of coal-tar oils on maggots. In each case six large maggots were dipped into the oil, immediately placed on blotting paper and then in glass vessels.

Reagent		2 hours	24 hours
Crude carbolic oil	motionless, 5 mins.	still; contracted	dead with brown patches
"Middle oil"	motionless immediately	,, ,,	dead, 2 brown, 4 yellow
Creosote oil	motionless almost at once	,, ,,	dead, slightly brown
"Heavy oil"	motionless in 2 mins.	,, ,,	dead, yellowish
Anthracene oil	moving freely in 10 mins.	still, but able to move head	All still, but heads capable of slight movement

By "still" we mean incapable of voluntary movement.

The first four oils killed these large maggots in a very short time, although they were placed in them only momentarily. Anthracene oil, by far the least effective, is the last fraction distilled from tar above 270° C., and is very deficient in phenolic bodies.

Six maggots were sprayed very lightly with creosote oil, immediately placed on blotting paper and then in a glass. Four of the six were motionless in two minutes. After two hours four were capable of moving. In six hours three were moving, and three were motionless, but capable of retracting their heads when touched. After 24 hours three were dead, two capable of retracting their heads, and one capable

TABLE XXIX.

Effects of fractions of crude carbolic, "middle" and "heavy" oils on large maggots. The same method was employed as in Table XXVIII. The controls were very lively when last observed after 48 hours.

Crude carbolic

		24 hours
(1)	Fraction up to 140° C.	3 pupated, 3 moving
(2)	,, 140—165° C.	3 retractile*, 1 dead, black, 2 dead, white.
(3)	,, 165—180° C.	4 dead, black, 2 dead, white
(4)	,, 180—187° C.	3 dead, black, 3 dead, brown
(5)	,, 187—197° C.	All dead with brown patches
(6)	,, 197—201° C.	All dead, soft, with brown patches

"Middle oil"

		Immediate	2 hours	24 hours
(1)	Fraction up to 174° C.	Motionless	Head brown, red patches on body	All brown-black and shrivelled
(2)	,, 174—184° C.	,,	Red patches on body	,, ,, ,,
(3)	,, 184—188° C.	,,	,, ,,	,, ,, ,,
(4)	,, 188—200° C.	Motionless	Red-brown	,, ,, ,,
(5)	,, 200—210° C.	,,	Red-brown patches	Blacker and more shrivelled
(6)	,, 210—220° C.	Almost motionless	,, ,,	Brown and slightly shrivelled
(7)	,, 220—230° C.	,, ,,	,, ,,	,, ,, ,,
(8)	,, 230—240° C.	,, ,,	,, ,,	,, and more shrivelled
(9)	Residue	Moving slightly in 5 mins.	,, ,,	3 dead and 3 retractile, flaccid, extended, not discoloured

"Heavy oil"†

		Immediate	15 mins.	4 hours
(1)	Fraction up to 200° C.	Motionless	All dead, contracted‡	Becoming red
(2)	,, 200—220° C.	,,	,, ,,	,, ,,
(3)	,, 220—240° C.	,,	,, ,,	,, ,,
(4)	,, 240—260° C.	Almost motionless	5 dead, 1 retractile	All dead
(5)	,, 260—280° C.	Not apparently affected	All retractile	Retractile
(6)	,, 280—300° C.	,, ,,	5 retractile, 1 moving	,,
(7)	Residue	,, ,,	Very lively	Retractile or moving

Creosote oil

		Immediate	45 mins.	3 hours
(1)	Fraction up to 170° C.	Motionless	Motionless, contracted	Contracted, not coloured
(2)	,, 170—200° C.	,,	,, ,,	,, ,,
(3)	,, 200—220° C.	,,	,, ,,	,, ,,
(4)	,, 220—240° C.	Almost motionless	,, ,,	,, ,,
(5)	,, 240—260° C.	Moving in 2 mins.	,, ,,	,, ,,
(6)	,, 260—280° C.	Moving rapidly in 2 mins.	Moving slightly	Retractile
(7)	,, 280—300° C.	Moving rapidly	Head retractile	,,
(8)	Residue	Lively	Very lively	Not moving, but irritable

* = capable of retracing the anterior end of the body when touched.

† = two minutes' exposure.

‡ = a condition like rigor and hard to the touch.

of moving slowly. Six others, which were not placed on the blotting paper, soon became motionless. All were dead and brownish in colour in 24 hours. We next proceeded to test the actions of various fractions obtained by distillation of crude carbolic, "middle" and "heavy" oils.

These experiments show that the potent constituents for killing maggots appear to be mainly contained in the fractions which distill over below 240° C.

Since other experiments had shown us that creosote oil was the best as a deodorant and preservative, we decided to investigate the effects of some of its constituents on maggots.

These experiments (Table XXX) show that in the absence of water the phenolic bodies are extremely toxic to maggots. Immediately on being taken out of the fluid the maggots become contracted, hard and tense. Very soon a red tinge appears in patches, and within 15 minutes they assume a deepened colour. After 24 hours they become black (cf. rigor in bodies, p. 178). The bases produce the opposite effect. The maggots remain white, but become extended and flaccid. The higher boiling fractions of the hydrocarbons are decidedly more toxic than those of lower boiling points. Each group of constituents of creosote oil possesses some degree of toxicity to maggots.

In another experiment, using maggots from another source, the bases caused a small percentage of deaths, but the fractions 190–220° C. and onwards of the hydrocarbons killed all the maggots treated. In considering these results the very short exposure should be borne in mind.

It may be seen from Table XXXI that in most cases momentary immersion in dilutions of the highly toxic constituents in water or inert fluids produces very little effect on the maggots. The presence of water appears to rob phenolic bodies of their toxic action to a large extent, for example 12·5 % phenolic bodies in water had no apparent effect. With more prolonged treatment maggots may be killed, if dilution is not carried too far.

In order to test this point further experiments were carried out on large maggots with emulsions (*A*, *B* and *C*) containing the cresols separated from creosote oil. Emulsion *A* contained 5 % of the phenolic fraction distilling over between 191–200° C. emulsified with the aid of 2 % of soft soap. Maggots kept in this emulsion for 45 minutes and then placed on blotting paper never appeared to be affected in any way, and pupated. Other maggots kept in the emulsion for 5 minutes were unaffected, and subsequently fed well for eight days on meat which was provided for them, and finally pupated. In another experiment

TABLE XXX.

Showing the effects of fractions of creosote oil on large maggots. Six large active maggots were used. They were dipped into the fluid and immediately placed on blotting paper, and finally in glass vessels.

	Immediate	1 hour	24 hours
Fraction of creosote oil from 170—220° C.	motionless	all dead, flaccid	brown
220—240° C.	,,	,, ,,	,,
Residue	—	just capable of moving	moving or retractile

Separated from creosote oil fraction 170—220° C.

Phenolic bodies	Immediate	2 hours	24 hours
Complete mixture	motionless almost immediately, red in 8 minutes	dead, hard, retracted, brown red	dead, black
Fraction 77—191° C.	motionless, red in 15 minutes	dead, hard, retracted, red	dead, black or black patches
,, 191—200° C.	,, ,,	,, ,,	,, ,,
,, 200—210° C.	motionless, tinged with red 15 mins.	,, ,,	dead, 5 black, 1 white
Residue	motionless quickly	retracted, less red	dead, black in patches

Bases	Immediate	2 hours	24 hours	3 days
"Water sol. fraction" (p. 159)	2 moving, 4 relaxed, motionless	extended, soft, retractile	2 dead, 2 retractile, 2 moving	3 dead, 3 pupating
"Water insol." ,,	2 motionless, relaxed	,, ,,	non-retractile, extended, flaccid	All dead
Hydrocarbon				
Complete mixture	—	2 motionless, 4 retractile	6 moving	4 dead, flaccid 2 pupae
Fraction 80—170° C.	—	1 motionless, 3 retractile, 2 moving	6 moving	2 dead, flaccid 4 pupae
,, 170—180° C.	—	1 retractile, 5 moving	6 moving	2 dead, flaccid 4 pupae
,, 180—190° C.	—	2 retractile, 4 moving	5 moving, 1 dead	1 dead, 5 pupae
,, 190—200° C.	—	6 slightly retractile, retracted	5 moving, 1 retractile	3 retractile 3 pupae
,, 200—210° C.	—	6 contracted	5 moving, 1 retractile	4 dead 2 pupae
,, 210—225° C.	—	6 contracted	3 moving, 3 retractile	3 dead 3 pupae
,, 225—240° C.	—	5 motionless contracted, 1 retractile	6 dead	6 dead, decomposed
Residue	—	4 motionless, 2 retractile	5 dead, 1 retractile	6 ,, ,,

	Immediate	2 hours	24 hours
Fraction creosote oil 170—220° C. 2 pts. ,, phenolic bodies 77—191° C. 1 pt.	motionless	dead, retracted, red	hard, black
Fraction creosote oil 170—220° C. 2 pts. Hydrocarbons, compl. mixt. 1 pt.	almost motionless	—	dead, soft, brown in patches
Fraction creosote oil 220—240° C. 2 pts. ,, phenolic bodies 77—191° C. 1 pt.	motionless	dead, retracted, red in patches	black, brown, hard
Fraction creosote oil 220—240° C. 2 pts. Hydrocarbons compl. mixt. 1 pt.	,,	—	dead, soft, brown

TABLE XXXI.

Further experiments with various extracts and emulsions of constituents of creosote oil. In each case six maggots treated.

	30 mins.	2 hours	24 hours
5 % aqueous emul. of fraction 170—220° C. + 1 % bile	no apparent effect	all moving freely	all moving freely
5 % aqueous emul. of fraction 170—220° C. + 2 % soft soap	,, ,,	,, ,,	,, ,, ,,
Aqueous extract containing phenolic bodies (0·63 % calculated as cresols)	,, ,,	,, ,,	,, ,, ,,
Aqueous solution 'calcium cresolate'* (0·91 % cresols)	,, ,,	,, ,,	,, ,, ,,
Mixture of equal parts hydrocarbon frac. 190—200° C. phenolic frac. 191—200° C.	still immediately; red in 6 mins.	dead, brown-red	black
Above mixture 1 part, medicinal paraffin 2 pts.	still almost immediately; contracted, red 15 mins.		dead, brown
Above mixture 1 pt., medicinal paraffin 9 pts.[1]	still 4 mins.		all alive third day
Above mixture 1 pt., medicinal paraffin 99 pts.[2]	no effect		,, ,,
Above mixture 1 pt., water 3 pts.†	35 mins. all moving		,, ,,
,, ,, 1 pt., ,, 9 pts.[3]	,, ,,		,, ,,
,, ,, 1 pt., ,, 99 pts.[4]	,, ,,		,, ,,
Medicinal paraffin (10 mins. exposure)	,, ,,		,, ,,
Olive oil (10 mins. exposure)	,, ,,		,, ,,

* Creosote shaken up with milk of lime and filtered. The filtrate contains the calcium salt of phenolic bodies equivalent to 0·91 %, calculated as cresols.

† Emulsified with the aid of bile salts.

[1] Exposure of one minute causes the maggots to become motionless and retracted; red tinge appears in 20 minutes, and all die.

[2] Exposure of seven minutes has no effect.

[3] Exposure of 30 seconds causes the maggots to become motionless and retracted; a red tinge appears in 20 minutes and all die.

[4] Exposure of seven minutes makes the maggots at first motionless and retracted, but they recover in 30 minutes. Even small maggots are not killed.

five small maggots were kept in the emulsion for 15 minutes. When placed on blotting paper they were motionless and flaccid. 20 minutes later they showed signs of recovery. A piece of meat soaked in the same emulsion was now placed in the vessel. Within 24 hours four of the maggots had completely recovered and were eating the meat. The other recovered later, and all were well and feeding on the eighth day. Finally all pupated, and flies emerged from the pupae. Maggots

kept for 15 minutes in water were little affected, and after feeding for a few days pupated. Maggots placed on fresh meat soaked in this emulsion attacked it immediately, never showed any signs of ill health and pupated on the 8th day. Flies emerged from all these pupae.

An emulsion, *B*, containing 2·5 % of the phenolic fraction distilling over between 191–200° C. and 2·5 % of the fraction distilling over between 200–209° C. emulsified with the aid of 2 % soft soap, and another emulsion, *C*, containing 5 % of the complete mixture of all the phenolic bodies (tar acids) contained in creosote oil were tested. During five minutes' exposure the maggots kept turning over, and on being removed were somewhat rigid. Within 24 hours all had recovered, and eventually pupated.

Not one maggot was killed by exposure to these emulsions[1].

Experiments were also carried out to test the action of creosote oil on maggots present in carcases.

The bodies of six guinea-pigs which had been dead ten days, and were much decomposed and contained innumerable maggots of all sizes, were placed in a large glass vessel and sprayed with 20 c.c. of creosote oil. A careful examination made 24 hours later showed that all the maggots were dead and black. In another case the carcase of a guinea-pig full of large maggots was sprayed with 5 c.c. of creosote oil. Within 15 minutes all the maggots were dead. Next day their bodies were dark red or black (Plate IV, fig. 9).

The body of a guinea-pig lying in the open and full of maggots was treated with 25 c.c. of creosote oil. On examination next day thousands of dead maggots were found in various parts of the carcase, but not a single living specimen.

Also the body of a goat was treated. Most of the thoracic contents and the greater part of the back had been eaten by maggots and very large numbers of maggots were present in these situations. About 400 c.c. of creosote oil were poured into those situations and over other parts of the carcase. Within an hour all the maggots seemed to be dead. Careful examination next day proved that all the maggots were dead and brown.

These and other experiments prove that by suitable treatment with creosote oil all the maggots present in a carcase can be killed.

The conditions under which creosote oil preserves carcases from the attacks of maggots are dealt with in the following sections.

[1] We have not had the opportunity of comparing the toxicity to maggots of pure ortho-, meta- and paracresol. (See experiments with the toluidines p. 114.)

Experiments designed to test the effects of treating small Carcases exposed in the open with various Tar Oils and other Reagents.

In the following experiments the carcases of freshly killed guinea-pigs were treated and left on the ground. All these experiments were started on 25 August. The bodies were examined almost daily.

1. Weight 315 grms. Intact. Skin treated with 15 c.c. of creosote oil, except head, anus and inner sides of thighs. 2nd day, some eggs on under side of head. 5th day, head almost completely eaten by maggots, and some eggs on anus. 8th day, head completely eaten and maggots seen on body under fore leg. No maggots found elsewhere. 15th day, skin intact wherever treated. Maggots had eaten the rest of the carcase, only bones and faecal material being found within the skin. *Remarks.* This experiment shows that the treated parts of a carcase are preserved from the attacks of maggots, but that eggs may be laid on untreated portions, and the maggots make their way into the body from such places. The necessity for thorough treatment of the skin and natural orifices is very clearly indicated.

2. Weight 365 grms. Intact. Whole surface of skin and orifices treated with 15 c.c. of creosote oil containing 2 % aniline. 3 c.c. injected into abdominal cavity and 1 c.c. into each pleural cavity. Every day for a fortnight, except when rain fell, water equivalent to one-eighth inch of rain was evenly distributed over the body and a circular area 8 inches in diameter. No eggs found up to the 15th day, when a few eggs found on nose, anus and thigh. On 22nd day a few scattered eggs on the coat. The body had a slight smell. 26th day, eggs had not hatched, and none freshly laid. 34th day, examination showed a little gray, pasty material on the under side probably arising from the exuding fluid. Smell slight. Few eggs, no maggots. On 49th day, condition similar. Skin of under side moist and tough. *Remarks.* In spite of frequent applications of water the body was well preserved for several weeks, and was never attacked by maggots. The few eggs deposited never hatched.

3. Weight 390 grms. Intact. Skin and orifices treated with 15 c.c. of creosote oil. The intestines of another guinea-pig were placed one inch from the body as a source of maggots. On 2nd day numerous eggs were present on the intestines, and on 3rd day hundreds of half-grown maggots. 5th day intestines almost eaten and numerous maggots wandering on them and on the surrounding ground. The carcase was quite free from eggs or maggots. 8th day, three large maggots brown and dead were found on the body, and a living maggot on the ear. These had migrated from the intestines. 14th day, no eggs or maggots. Body dissected and found to be excellently preserved. Muscle looks normal, peritoneal surfaces glistening and intestines well preserved. Abdominal walls not green. Slight rancid smell which disappeared quickly on exposure to air. *Remarks.* Surface treatment alone kept the body in an excellent state of preservation for a fortnight. No eggs were deposited on it, and no maggots migrated on to the body, in spite of large numbers being present in the intestines close by.

4. Weight 360 grms. Abdomen and thorax opened. External surfaces of the body and serous cavities treated with 25 c.c. of creosote oil, containing 5 % aniline. 5th day, an egg found on the lip. 8th day, same egg unhatched. 15th day, a few eggs on the nose. 22nd day, a few scattered eggs. 26th day, condition excellent. 34th day, same. 49th day, many eggs on surface, but no maggots seen. A moist patch on the skin underneath. Smell very slight. *Remarks.* Even though the body was opened it was well preserved by superficial treatment for seven weeks. Towards the end of this period eggs were deposited but no maggots developed from them, and a very slight amount of putrefaction had occurred as shown by the smell.

5. Weight 380 grms. Abdomen and thorax opened. Skin treated with 20 c.c. of creosote oil, and orifices and serous surfaces with 12 c.c. No eggs deposited up to the 8th day. Two eggs found under nose on the 15th day. On 22nd day a few scattered eggs. Condition excellent. 26th and 34th days, same. 49th day, skin and intestines very well preserved. Very slight smell. Numerous eggs but no maggots on the body. *Remarks.* This is another example of an opened body very well preserved by superficial treatment for seven weeks. Again though eggs were deposited towards the end of this period no maggots developed from them.

6. Weight 394 grms. 19 c.c. of creosote oil containing 2 % aniline used in treatment of skin and orifices. Then abdominal and thoracic cavities opened and treated with 30 c.c. of 10 % hydrochloric acid. On 5th day, five small patches of eggs were found on the intestines. 8th day, the eggs had not hatched. 15th day, most of the eggs had not hatched, but a few large maggots were found in the body, but none on the oil treated parts. 22nd day, numerous half-grown maggots in the abdomen. None on surface. 26th day, the maggots have eaten a large part of the thorax, but have not touched the skin. 34th day, skin and intestines well preserved, but contents of the thorax completely eaten. *Remarks.* The parts treated with creosote oil were well preserved, but maggots made their way into the body from parts not so treated. (See No. 1.)

7. Weight 390 grms. Skin treated with 16 c.c. of creosote oil and 2 % aniline, orifices with 2 c.c. Abdominal cavity opened and 2 c.c. injected into stomach, and 3·5 c.c. into coecum. Exposed serous surfaces not treated. 5th day, no eggs; intestines appeared normal. 8th day, a few eggs on the lips and near the abdominal wound. 15th day, eggs on anus and in mouth. None on the intestines. 22nd day few scattered eggs on surface. 26th day, no freshly laid eggs. Hair loose underneath. 34th day, few eggs, no maggots, very well preserved. 49th day, many eggs; no maggots; very slight smell; very well preserved. *Remarks.* The result of this experiment is most interesting, but in the absence of further experiments of this nature we are not in a position to make important deductions from it.

8. Weight 370 grms. Stomach and intestines removed and the cavity filled with hay and abdominal walls approximated with sutures. Skin treated with 20 c.c. of creosote oil and orifices with 8 c.c. Up to the 15th day no eggs deposited. 22nd day, a few scattered eggs. 26th day, very well preserved. No eggs. 34th day, a few eggs, but no maggots. 49th day, eggs had been deposited, but the body was well preserved. *Remarks.* There seems to be no advantage gained by removing the stomach and intestines.

9. Weight 375 grms. Abdominal and thoracic cavities opened and serous surfaces and natural orifices treated with 9 c.c. of creosote oil and 2 % aniline. Skin not treated. The carcase was laid on its side on a patch of ground painted with the same solution. 2nd day, seven eggs on chest. 5th day, few eggs on the chest and back. 8th day, numerous small maggots in skin over lumbar region. They had made a large hole through the skin. No maggots between the skin and the ground, nor on intestines or head. 15th day, many maggots under head and in eye and mouth. Several holes through the skin of the back. No living maggots on the intestines, though some dead maggots were found amongst the intestines and on the ground. 22nd day, the muscular tissues had been eaten by maggots. *Remarks.* This experiment again shows the necessity for complete surface treatment.

10. Weight 371 grms. The carcase of an animal dead 48 hours but apparently without eggs or maggots, was sprayed so as to moisten the whole surface with 12 c.c. of creosote oil. On 2nd day no fresh eggs laid on it, but medium sized maggots, dead and brown, were protruding from the mouth. These were apparently in the body before treatment. During the next five days no fresh eggs were laid. 13th day, a few fresh eggs but no maggots. 17th day, a large hole was seen in the abdomen and large maggots inside the carcase. These no doubt were present in the carcase before treatment. Skin well preserved. 26th day, internal organs partially eaten by maggots; skin well preserved. 40th day, no maggots now on the carcase. The internal parts much decomposed. Skin well preserved. *Remarks.* Spraying into the mouth failed to kill some of the maggots present in the body. These maggots devoured as much of the body as they could, but no fresh maggots from eggs laid on the body after spraying attacked it.

11. Weight 390 grms. Intact. Skin and orifices treated with 20 c.c. of "heavy oil." 5th day, no eggs. 8th day, carcase distended with gas, no eggs. On 12th day, no eggs. The body was opened. The muscles looked fairly well preserved, but gas was found between the muscles, and in the abdominal cavity. Peritoneal surfaces not glistening, though intestines well preserved. Abdominal muscles greenish. Slight putrid odour. Hair comes off over abdomen. *Remarks.* This carcase, although moderately well preserved, was not so well preserved as No. 3. It should be noted that both bodies were of the same weight, and 20 c.c. were used in the treatment of No. 11, and 15 c.c. in the treatment of No. 3.

12. Weight 380 grms. Intact. Skin and orifices treated with 20 c.c. of "heavy oil" containing 2 % aniline. The carcase was daily treated with water in the same way as No. 2. No eggs were found up to the 8th day. 15th day, some scattered eggs were found on the head, ears and thigh. On 22nd day, hair loose underneath. Slight smell. 26th day, no maggots or fresh eggs. 34th day, gray, pasty material on under side. Few fresh eggs. 49th day, condition similar to No. 2. *Remarks.* The "heavy oil" exerted a similar influence to creosote oil under similar conditions as may be seen by comparing Nos. 2 and 12. The former was however the better preserved of the two.

These experiments show that in warm and showery weather, particularly favourable for rapid putrefactive changes, at the height of

the fly season the bodies of small animals can be well preserved for several weeks by efficient surface treatment with coal-tar oils, especially creosote oil. Flies are deterred from laying eggs for a considerable time, and though finally many eggs may be deposited maggots seldom develop from them. In order to obtain such results surface treatment must be thorough for eggs may be deposited on untreated areas and maggots developing from them make their way into the carcase from these areas. Even opened carcases can be similarly preserved from putrefaction and the attacks of maggots, if the exposed surfaces are treated. No advantage is obtained by removing the stomach and intestines. Maggots do not migrate on to treated carcases, even though their supply of food is exhausted.

The influence of creosote oil extended over several weeks even though the bodies were frequently wetted by rain, and some of them wetted with water daily during the first fortnight. Dissections showed how efficiently treatment with creosote oil protected the bodies from the influence of rain and soil water.

It was particularly noticeable that while untreated carcases were covered with flies, very few alighted on the treated carcases, even after three weeks, and these never remained long on them.

Our experiments with small bodies had given such satisfactory results that we decided to test the actions of various reagents on the carcases of larger animals, such as goats. In all cases the carcases were laid on the ground on their right sides in the middle of a large lucerne field, and lightly covered by sacking supported by hurdles.

Goat I. Weight 25 lbs. Killed 20 August. 48 hours later, when a few eggs had been deposited, 270 c.c. of double strength "Solution B" (p. 120) were injected into the femoral artery, and the skin and orifices treated with 900 c.c. of the same solution. On the 1st day after treatment many eggs were found on the lips, flank and anus. On the 3rd day many more eggs had been deposited, and many flies, mainly *Lucilia*, were visiting the carcase. On 4th day eggs were very numerous, but no maggots were seen. The abdomen was slightly distended, subcutaneous emphysema was present in places, and the skin was green. On the 6th day, wound, mouth, and eyes were free from eggs. Many small maggots were present on the under side and between the thighs. The eggs laid on the upper side had not hatched. 8th day, condition similar, but superficial epidermis of legs becoming loose. 11th day, hair loose except in the driest parts and maggots on the skin; small maggots very numerous underneath and round the anus. 19th day, almost all the maggots are dead. The flesh does not seem to have been eaten. Smell slight. On cutting into it the thigh muscle was found to be well preserved. 21st day, very few maggots on the surface, though some seemed to be present under the skin. 24th day, beetles have made shallow holes through the skin. A few sluggish maggots

found underneath the body. 36th day, numerous small maggots in certain areas of the body. 43rd day, some half-grown maggots present in the thorax, and mouth. The exposed thigh muscle was very much decomposed. 59th day, the body disintegrating and numerous maggots in the thorax and round the anus. 107th day, the remains of the body appeared to be in the same condition as on the 59th day.

Remarks. Although innumerable eggs were laid on the carcase and maggots developed from them they grew very slowly and hardly any survived. Even though some of the maggots eventually attained a large size they were sluggish and matured slowly. Putrefaction was delayed to some extent. The effect of this treatment is best seen by comparison with the control Goat VIII.

Goat II. Weight 25 lbs. Killed 20 August. 46 hours later 90 c.c. of crude carbolic acid were injected into the femoral artery, and the skin and orifices treated with 800 c.c. of "Solution B." On the first day after treatment eggs were deposited on the flank and round the anus. On 3rd day many more eggs on legs and abdomen, but not elsewhere. Wound free. 4th day, very small maggots found near the ground, but not elsewhere. Skin green in places and some subcutaneous emphysema. 6th day, numerous very small maggots in wound and on under surface of the body. 8th day, hair loose near ground, and many small maggots on the skin in these regions. 19th day, a large hole had been made into the thorax, and innumerable maggots were seen in the lung, in femoral wound and under the thigh, where they had eaten through the skin. 22nd day, the maggots had made further progress. 24th day, most of the thorax and a large part of the hind quarters had been eaten by maggots. 400 c.c. of creosote oil were poured on to the body and into the holes caused by the maggots. About one hour later the maggots were found to be dead. 27th day, all the maggots were dead and brown, except a few on a spot on the leg which had not been treated. 36th day, no living maggots seen. 44th day, no living maggots seen. There was no smell, and the treatment with creosote oil seemed to have completely preserved the remains of the body. On the 59th and 107th days, the body seemed to be in the same condition.

Remarks. The early skin treatment delayed the growth of the maggots to a considerable extent, and the injection seems to have delayed putrefaction, but no apparent difference was made to the maggots when they penetrated into the tissues. The further treatment with creosote oil killed the innumerable maggots present in the carcase, prevented eggs from being deposited subsequently and completely checked further putrefactive changes.

Goat III. Weight 122 lbs. Killed 20 August. Treated 28 hours after death. 560 c.c. of a fluid consisting of commercial formalin one part and 10 % aqueous solution of sodium chloride four parts injected into the femoral artery. Skin and orifices treated with 2000 c.c. of "Solution B 1." On the 1st day after treatment no eggs or maggots were seen. 2nd day, innumerable eggs in coat over abdomen and patches in mouth and near the wound. 4th day, numerous flies on the carcase, which smells considerably. Skin becoming green. Many more eggs have been deposited on the back of the neck and near the ground, and there were thousands of small maggots near the wound. 5th day, many maggots of the same size in other situations, and a small hole had been made through the skin near the anus. Skin very green, and the body greatly distended with gas, though the hair was not loose.

Owing to the condition of the body the skin was treated with 350 c.c. of creosote oil, and the gas let out by small openings into the peritoneal and pleural cavities, and through each hole thus made 50 c.c. of creosote oil were poured. 7th day, all maggots dead, and smell slight. On 9th and 12th days, no eggs or living maggots seen. Smell hardly perceptible. On 19th day, the body seems very well preserved; slight rancid smell. 25th day, some very small maggots near the ground. The body now smells more than before. 200 c.c. of creosote oil were put into the cavities and 200 c.c. on to the hair touching the ground, without moving the body. 28th and 37th days, no living maggots seen. 45th day, no living maggots seen; some smell. The body can be dragged along the ground by the horns. 60th day, no living maggots or fresh eggs seen. Slight smell. Skin moist underneath. 107th day, no visible change. The body lay in the field without further treatment and unprotected till 27 October, 1916, a period of 14 months. At this time the body was opened, found to be well preserved, and burnt.

Remarks. The injection of formalin did not prevent gas formation or discoloration of the skin, changes which are preliminary to disintegration (p. 179). Subsequent treatment with creosote oil killed the maggots and, though there was some smell while the gases from the emphysematous tissues were escaping, the body was so well preserved that it remained on the ground without apparent change for a year.

Goat IV. Weight 22 lbs. Killed 20 August. Treated 28 hours after death. 110 c.c. of a fluid consisting of one part commercial formalin and four parts 10 % aqueous solution of sodium chloride injected into the femoral artery, and the skin and orifices treated with 190 c.c. creosote oil containing 2 % aniline and 1 % ox bile.

On the 1st, 2nd and 4th days, the body was observed for some time and no flies were seen to approach it and not a single egg was found on it. The surface was oily. After 4th day, the covering of sacking was removed. On 5th day, no eggs or maggots were seen. Skin not green; surface moist with oil. 7th day, no eggs or maggots seen, a few flies alighting on the upper drier surface. 9th day, a few eggs on neck, none seen elsewhere; a few flies. 12th day, not distended. A few dead brown eggs on neck. No living maggots. Hair not loose. 20th day, very numerous eggs on back, neck, thigh and near ground. A few tiny maggots on upper shoulder. Skin is now not moist with oil on the upper surface. 23rd day, maggots have grown slightly on back and neck. The body could be lifted by one leg. 400 c.c. of creosote oil put on the skin. 28th and 37th days, no living maggots seen. 45th day, no living maggots seen; slight smell. 60th day, no maggots and no signs of decomposition, but a few eggs on the back. 107th day, condition same. The carcase remained in much the same condition during the next year.

Remarks. In the early period the condition of this carcase was better than that of No. III. No eggs were deposited on it for nine days. In both cases the original treatment was the same, but while in No. IV the skin treatment with creosote oil was carried out the day after death, in No. III this was not done till the 6th day. It should be noticed, however, that in relation to its weight the surface of No. IV was much greater than that of No. III and more creosote oil was applied per unit weight.

Goat V. Weight 76 lbs. Treated 28 hours after death. 150 c.c. of creosote oil were injected into the femoral artery, and 45 c.c. of creosote oil containing 2 %

aniline and 1 % bile into the subcutaneous tissues in several places, the sides of the neck, flanks and proximal parts of the limbs, and into the abdominal and right pleural cavities. The skin and orifices were treated with 350 c.c. of the same solution.

On the 1st and 2nd days no eggs were found and it was noticed that flies did not approach the body. 4th day, abdomen distended, but no eggs have been deposited. 5th day, skin turning green; no eggs found. 7th day, much distended; skin green near thigh wound. Some eggs in left nostril. 12th day, a few eggs on skin, lip and tongue. The eggs seen previously in the nostril have become brown and shrivelled. No maggots seen. 20th day, a few very small maggots in wound. Many eggs near the ground. The eggs previously noted appear to be dead. 25th day, a very few small maggots near ground, but none elsewhere. Some smell. Small openings were made into the thorax and abdomen to let out the gas. The organs appear to be in an excellent state of preservation. 450 c.c. of creosote oil put into these openings and 350 c.c. on to the skin. 28th, 37th, 45th and 60th days: no living maggots found. Very slight smell. 107th day, same. The body was left on the ground without further treatment until 27 October, 1916, 14 months in all, and on dissection was found to be very well preserved.

Remarks. The early phenomena of distension and green discoloration of the skin were again evident, but the body remained for 14 months in a remarkably good state of preservation.

Goat VI. Weight 65 lbs. Killed and treated on 7 September. The skin and orifices were treated with 1000 c.c. of creosote oil and 100 c.c. were injected into the thoracic and 150 c.c. into the abdominal cavity. 3rd day, distended with gas; no eggs found. 5th day, no eggs or maggots found, but flies were seen resting on the carcase. 8th day, a few eggs on the lip, and some very small maggots on the anus. On enlarging the injection hole some gas escaped from the abdominal cavity. Some smell. Upper surface treated with 200 c.c. of creosote oil, and 50 c.c. poured into the peritoneum. 11th day, very numerous eggs on the under surface of the head, which is moist. Few small living maggots on the lower eye, and some, which are discoloured and appear to be dying, on the chest. Many eggs on back near the ground. 28th day, most of the eggs on the skin are dead, but a few large living maggots were found on a portion of the gut which was protruding. 43rd day, flies visiting the carcase. A few small maggots in the mouth. Numerous dead eggs on the chest. Some maggots still alive on the protruding gut. 91st day, little change. A year later (26 October, 1916) the carcase was entire, but dropped to pieces when raised with a fork.

Remarks. On comparing this carcase with No. V after 13 months' exposure the beneficial effect of the original injection of the blood vessels with creosote oil, combined with the skin treatment, is thrown into relief.

Goat VII. Weight 60 lbs. Killed and treated on 9 September. The abdominal organs were removed and placed on the ground about four feet from the carcase. The cavity packed with hay, and the abdominal walls roughly approximated with string sutures. Skin, orifices and abdominal wound and hay treated with 1000 c.c. of creosote oil. On 3rd day, no eggs found, and no flies on the body. 5th day, no eggs and no maggots. 8th day, no eggs found. 11th day, a few eggs in mouth and nostrils, but none elsewhere. 20th day, many eggs under head and udder. No

maggots found. On 28th day, innumerable eggs on the head, and some tiny dead maggots near them. Some dead eggs on anus. The thin abdominal walls now mummified. 43rd day, eggs under head dead, and no living maggots found except under the chin and on the lower eye. On the 91st day, condition similar. A year later (26 October, 1916) the carcase was entire but dropped to pieces when raised with a fork.

Remarks. This carcase was only treated once with creosote oil. A few maggots developed in some situations, but very few survived and these made very little progress. Evidently the carcase provided little, or no, food for maggots. No advantage seems to have been derived from the removal of the abdominal organs.

Abdominal organs of Goat VII. 100 c.c. of creosote oil were distributed over the exposed surfaces. 3rd day, exposed surfaces dry and flies walking over them, but no eggs found. No smell. 6th day, some small maggots at one side, but the greater part of the mass was free from maggots. 8th day, some small maggots at the place mentioned, but not elsewhere. The rest of the surface dry and hard. 200 c.c. sprayed on the area where the maggots were present. On 11th day all maggots dead. 20th day, a few eggs found. On 28th day, the surfaces like parchment. The whole mass was carefully examined and no maggots found. No smell. 43rd day, well preserved, numerous dead eggs in two situations. 91st day, condition similar.

Remarks. This experiment shows that, contrary to the popular idea, the intestines are more easily preserved by surface treatment than other portions of the body (p. 206).

Goat VIII. Control. Weight 33 lbs. Killed on 20 August, and exposed 28 hours after death. On 2nd day, hundreds of flies were sitting on the carcase, and innumerable eggs had been deposited on the body. On 3rd day, flies numerous, and countless eggs everywhere, and innumerable small maggots in the coat, on the anus, eye, mouth, nose, groin and scrotum. 5th day, stench intolerable; flies numerous. Maggots have eaten through the abdominal walls, and the intestines have ruptured. Numerous half-grown maggots found under the carcase and elsewhere, except where the skin was dry. Hair very loose. 6th day, flies numerous; abdominal cavity full of half-grown maggots; head half eaten. 8th day, a hole a foot in diameter over the thorax was seen, full of maggots. Innumerable maggots of all sizes on the skin. 10th day, the whole carcase was eaten except the fore legs, and part of the neck, where very large numbers of very large maggots were at work. 12th day, nothing left except hair, bones, horns, hoofs, some fibrous tissue and stomach contents.

Remarks. The great power of creosote oil in repelling flies was very evident when comparing the numbers found on this and on the treated carcases. The carcase was completely eaten by the maggots within 11 days, while in creosote treated carcases hardly an egg had been deposited in that time.

Conclusions from experiments on the carcases of goats.

The control attracted large numbers of flies of many kinds, and was reduced to a skeleton in eleven days, smelling intolerably during the process.

Treatment with "Solution B" produces very great mortality amongst the eggs, and also prevents the development of such maggots as do hatch, but possesses small value as a repellent for flies, and only delays putrefaction to a small extent. A carcase efficiently treated acts as a trap for the destruction of eggs and small maggots on a large scale for a variable period of time. The time this treatment remains operative depends mainly on the rainfall, since the potent constituents are letched away, and the carcase left without protection. The remains then become available as food for maggots. By surface treatment with creosote oil flies are repelled almost completely for a week, and to a smaller extent for a long period. The deposition of eggs on carcases treated with creosote oil after two or three weeks has certain advantages. A very large proportion of the eggs shrivel and never produce maggots. Great numbers of the maggots which do emerge die within a few days, and in properly treated bodies it is doubtful whether any reach maturity, and give rise to flies. Eggs continue to be deposited for a long time. Carcases in this stage therefore act most efficiently as agents for destroying the coming generations of flies and diminishing the total fly population. Apart from these effects surface treatment with creosote oil cuts off the access of water from any source, the potent constituents are not extracted, the skin is made leathery and the internal organs preserved for a long time. The combined effect of surface treatment with injection preserves the body for many months. As in the case of small carcases distension with gas, and green discoloration of the skin though suggestive of putrefaction, are not followed by disintegration in properly treated carcases. At any stage of decomposition maggots may be destroyed, smells eliminated and the process arrested by suitable treatment with creosote oil.

The stomach and intestines, whether in or out of the body, can be more easily preserved than other tissues by treatment with creosote oil. We are inclined to the opinion that the removal of the abdominal organs is disadvantageous, for intact carcases are well preserved by surface treatment, and the procedure of removal permits of the introduction of organisms into the exposed tissues.

Fly Repellents.

Throughout all our experiments, some of which extended over long periods, we compared as well as we could the numbers of flies approaching, alighting on and walking over treated carcases and untreated

controls. It is impossible to record these observations in precise terms, and we can only state that some of the coal-tar oils exert a very marked and prolonged repellent action, the period apparently depending on the rate of evaporation. We attempted to increase this action by varying the proportion of the constituents and by adding to creosote oil small quantities of reagents, which possess well marked repellent powers and are soluble in creosote oil, such as aniline, bone oil, pyridine and various other bases. Of these the most satisfactory appear to us to be the bases derived from "light oil" which add very distinctly to the repelling and other properties of creosote oil without appreciably affecting its flash point or pleasant smell. In the treatment of faecal material and in deterring flies from entering habitations and approaching food it is desirable that the greatest possible deterrent effect should be exercised.

For general use as an inhibitor of putrefaction, deodorant, repellent of flies, and destroyer of maggots we recommend the addition to creosote oil, of the type we have previously described (p. 123), of sufficient bases derived from "light oil" to make the proportion of phenolic bodies to bases two to one. This mixture has been called "Solution C."

If a means could be devised for preventing biting and other flies from settling on the exposed parts of the body, such as the hands, face and neck it would add immensely to health and comfort in tropical and subtropical countries. For this purpose an accurate knowledge of the relative repellent powers of various reagents, alone and in solution, is necessary. In the study of the relative powers of disinfectants descriptive methods failed to give reliable means of comparison. The failure of descriptive methods was even more evident when attempting to determine the relative powers of repellents. We have devised an apparatus with which we hope to obtain results of sufficient accuracy to make a reliable comparison of repellents possible.

The repellent powers of a substance appear to depend upon its nature and rate of evaporation, the latter affecting the length of time the repellent remains operative. Some of the best repellents are poisonous, inflammable or very volatile. These properties may be submerged by dissolving suitable quantities in such fluids as creosote oil. By such a procedure the high flash point of creosote oil may not be appreciably affected. The evaporation of the added substance is so retarded that it remains operative for a considerable time. To prevent flies from alighting on the exposed skin repellents might be employed in two ways: by application to (1) the clothing and (2) the skin. Clearly for these purposes irritating, poisonous and inflammable reagents must be avoided.

Conclusions.

1. In summer time maggots play a very important part in the destruction of exposed carcases.

2. Coal-tar oils, such as crude carbolic, "middle," creosote and "heavy" oils, when used at full strength kill large maggots almost immediately. Of these the most suitable for general use is creosote oil. Anthracene oil is much less effective.

3. The most potent constituents are contained mainly in the fractions which distil over below 240° C.

4. Each group of constituents of creosote oil possesses some degree of toxicity to maggots even when the exposure is momentary. The phenolic bodies in the absence of water are extremely toxic to maggots, which immediately become contracted, hard and tense, and within 15 minutes assume a deep red colour. The bases are also toxic, but the maggots remain white, and become extended and flaccid. The higher boiling fractions of the hydrocarbons are decidedly more toxic than those of lower boiling point.

5. Momentary immersions in dilutions of highly toxic constituents in water or inert fluids produce little effect on maggots. The presence of water seems to enable maggots to resist the toxic action of phenolic bodies to a large extent. By more prolonged treatment, however, the maggots may be killed, if dilution is not carried too far.

6. Maggots can survive 15 minutes' immersion in emulsions containing 5 % phenolic bodies and reach maturity. They can feed on meat soaked in such emulsions without ill effect.

7. Maggots present in carcases are killed by suitable treatment with creosote oil.

8. Even in warm and showery weather, particularly favourable to putrefactive changes, at the height of the fly season, the bodies of small animals can be well preserved for several weeks by efficient surface treatment with coal tar oils, especially creosote oil.

9. To obtain such results the surface treatment must be thorough, for eggs may be deposited on untreated areas and make their way into the carcase from these areas.

10. Open carcases can be similarly preserved, if the exposed surfaces are treated.

11. This treatment protects the bodies from the influence of rain and soil water.

12. Experiments on the bodies of goats show that large carcases can be preserved in the same way as small ones.

13. The combined effect of surface treatment with injection preserves the body for many months.

14. The removal of the abdominal organs is disadvantageous for intact carcases are well preserved by surface treatment, and the process of removal permits of the introduction of putrefactive organisms into the tissues.

15. Surface treatment with watery emulsions, such as "Solution B," delays putrefaction to a slight extent, but causes the destruction of innumerable eggs and small maggots. Sooner or later rain letches away the potent constituents and the carcase, left without protection, becomes available as food for maggots.

16. On the other hand treatment with creosote oil repels flies almost completely for a week or two, and to a less extent for a long period. After two or three weeks eggs are deposited. A large proportion of these die, and great numbers of the maggots, which emerge from them, also die. In fact in well treated carcases it is doubtful if any maggots reach maturity. Hence in their different ways carcases treated with such fluids as "Solution B" or creosote oil act as traps for destroying fly eggs and maggots.

17. At any stage of decomposition maggots may be destroyed, smell eliminated and the process arrested by suitable treatment with creosote oil.

18. Several reagents, possessing fly repellent properties, can be dissolved in creosote oil, in order to increase its efficiency in this respect. Of these the most satisfactory are the bases derived from "light oils." These increase the repelling and other properties of creosote oil without appreciably affecting its flash point or smell.

19. For general use as an inhibitor of putrefaction, deodorant, repellent of flies and destroyer of maggots we recommend the addition to creosote oil, of the type described (p. 123), of sufficient bases from "light oil" to make the proportion of phenolic bodies to bases two to one.

20. The study of fly repellents by methods sufficiently accurate to make reliable comparisons possible might suggest means for preventing flies from alighting on exposed surfaces of living persons.

Part IV. The Control of Nuisances due to Flies and Putrefaction.

Our work was undertaken for the purpose of devising methods for overcoming the dangers and nuisances arising from flies and putrefying substances, and throughout we have kept this aim steadily in view. In this part of our paper we mention only those experiments which illustrate practical methods for dealing with large carcases, maggots, manure, fly infested habitations, etc. As far as possible we have quoted the impressions of competent observers, who watched our experiments or independently tested our methods.

Experiments on the Carcases of Horses.

Two horses, a white (*A*) and a black (*B*), were killed at 8 a.m., on 7 September, 1915, and allowed to lie where they fell in an open field. They were treated at 4 p.m. in the presence of Col. C. H. Melville, A.M.S., who visited them frequently during the next two months, often invited officers who might be interested to see them, and kept his own notes.

One horse, (*A*), was injected through the carotid artery with 2 gallons of creosote oil[1], the process occupying a few minutes. Then the fluid was applied in small quantities at a time on to the body from a watering can fitted with a rose and distributed with a coarse brush in the direction of the hair. The *whole* surface of the body was treated in this way. A small quantity was poured into the eyes, ears, mouth and anus. About half a gallon was used in the external treatment.

The other horse, (*B*), was not injected, but about half a gallon was poured through openings into the pleural and peritoneal cavities, after cutting the intestines to allow the gas to escape. A large wound was made in the right thigh, and the whole surface treated in the same way as horse (*A*). About one gallon of the fluid was used in the complete treatment, which occupied less than 15 minutes. "There was an enormous number of fly eggs, probably enough to fill a pint measure, near the mouth and in sheltered positions, laid already in the eight hours that elapsed since death." To hide the bodies from the inquisitive, hurdles were leant against them and a few old sacks thrown over them. In both carcases gas had developed in large quantities in the intestines between the time of death and treatment.

[1] Most of the experiments mentioned in this part were carried out with the creosote oil mixtures, especially that recommended on p. 199.

The results of these experiments may be stated in the words of Col. Melville. "The two horses were allowed to lie out in a field, covered with sacks, for 29 days. They were visited from time to time, but no important changes occurred."

On 15 September 600 c.c. were poured into the peritoneum, 400 c.c. into the thorax and 400 c.c. sprinkled on to the skin of the black horse (*B*). The white horse (*A*) was not treated. On 18 September creosote oil was sprayed on the sacks covering the black horse, and some also on the blood patches on the ground near the head, which had not previously been treated. About 1400 c.c. were used in this manner. On the same day the sacks covering the white horse were sprayed with 1000 c.c.

During this period very few flies visited the carcase of the white horse (*A*) and only a few scattered eggs were deposited on it. There was no smell at any time and no subcutaneous emphysema.

In the carcase of the black horse (*B*) gas developed, subcutaneous emphysema was present and the gas gradually escaped through the incisions we had made. The escaping gases probably contributed to the slight smell, and accounted for the considerable number of flies attracted to this carcase after the first week. Many eggs were deposited on a piece of intestine which protruded through the opening made in the abdomen, and some maggots developed from these. Thousands of eggs were deposited on untreated blood patches near the carcase, on the hairs of the tail and on the untreated sacks.

"On 6 October, 1915 (the horses having been killed and treated on 7 September), various dissections were made as follows:

White horse (*A*). The skin was removed from the left gluteal region and as far down as the hock. The fat and fascia were also removed and the various muscles defined. The flesh was firm and normal in appearance; no signs of putrefaction anywhere. The colour of the muscle was slightly different from that of fresh muscle, and there was a very superficial change of colour on exposure to air.

The abdomen was freely opened and the intestines allowed to escape. There was no sign of putrefaction and the peritoneal surfaces retained the gloss present in fresh tissues. The spleen, stomach and lungs showed no change. The skin was then reflected from the sides of the face; there was no sign of change here, nor in the muscles of the neck."

We may add that the hair was loose on the under side of the abdomen, and here and in the neck the tissues were oedematous. The skin was very tough and the abdomen not distended with gas. No unpleasant

odour was noticed during the dissection, though the intestines had their characteristic smell.

"A similar series of dissections was made in the case of the black horse (*B*) with identical results. The flesh in this case showed absolutely no change in colour or consistency, and could have passed anywhere for fresh butcher's meat except for a slight rancid smell."

We may add that the neck muscles were oedematous, and there was some rancid smell when the dissection was being made.

"It will be remembered that this horse was not injected with creosote oil as in the case of the white horse; the fluid was merely brushed over the surface of the body and some poured into the thoracic and abdominal cavities, about a gallon in all being expended. The secondary applications to the sacks and blood patches on 18 September undoubtedly kept flies off the carcase, but did not in any way, in my opinion, influence the question of putrefaction. This is a matter of considerable importance, as the technique of application demands no apparatus more recondite than a watering can and brush. No skill is required, and the time necessary to treat a horse completely is between ten minutes and a quarter of an hour, with two men working. Injection, however, is also an easy and rapid process and very satisfactory, though requiring rather more of the fluid."

Fleet-Surgeon D. W. Hewitt, R.N., representing the Admiralty, visited Cambridge on 23 September, and saw a number of experiments then in progress. The Medical Director General of the Navy, Sir Arthur W. May, K.C.B., most kindly permitted us to read the reports he had received, and to make quotations from them. The following extracts are from Fleet-Surgeon Hewitt's report:

"These experiments have undoubtedly been governed by a great deal of care and forethought, in several instances 'controls' have been carried out at the same time. The bodies examined were those of guinea-pigs, rabbits, goats, pigs and horses, grouped in three different areas, separated by from two to three miles....They had all been exposed to the open air, rain, and sunshine for periods varying from a fortnight to six or seven weeks, thus as far as possible simulating the actual conditions met on a battle area.

The methods of treatment may be described under three headings:

(*a*) The fluid brushed over the whole surface of a dead animal.

(*b*) A combination of (*a*) with injection into the carotid artery.

(*c*) A combination of (*a*) with injection of the fluid into openings in the peritoneal and pleural cavities.

I was shown carcases stated to have been treated by each of the above methods, and came to the following conclusions:

The bodies in either case were all deodorised, putrefaction was greatly retarded or prevented, and the fly maggots were all killed.

The most effective of all the above methods was (*b*); and the body of a horse treated thus a fortnight ago was fresh and without odour. Process (*c*) was also efficient, and there were numerous flies round the body of a horse so treated, but no smell or maggots. Process (*a*) was effective as regards guinea-pigs and small animals, and would probably suffice for a human body."

Professor J. Stanley Gardiner, who inspected the bodies on 17 December, more than three months after the death of the animals, was particularly struck by the absence of smell and the excellent appearance of the meat, when an incision was made into the body.

The carcases of these two horses were allowed to lie without further treatment in the field for 12 months after the dissections described, and were examined from time to time.

White horse (*A*). In the exposed meat a slight very superficial change in colour occurred, and the surface became soft. Moulds grew on the exposed intestines. During the winter the carcases were often very wet, but there was very little smell at any time.

On 20 April, 1916, seven and a half months after death, the carcase was shrunken, but there was no smell, and on section the larger muscles were very firm and compact, but otherwise normal in appearance, and the smaller exposed muscles very tough and the colour of mahogany. The appearance of the left gluteal muscle may be seen in the accompanying photograph (Pl. V, fig. 10). Microscopically the muscle fibres were very well preserved, the striae being exceedingly evident. Very few of the fibres were degenerated (Pl. V, fig. 12). Sections stained by Weigert's method showed in places minute slits in the connective tissues with numerous undegenerated non-sporebearing bacilli at the margins. It is probable that these organisms, which may have been introduced into the blood stream when the animal was shot, were present at the time of injection and were killed and fixed *in situ*.

On 23 May, 1916, eight and a half months after death portions of meat were taken from (*a*) the left shoulder, (*b*) the left gluteus, and (*c*) the upper part of the right thigh for chemical analysis. The meat from the left shoulder was easy to grind with sand and had an unpleasant smell, suggestive of acetamide; that from the left gluteus was dry and not easy to grind and smelt of creosote oil, while that from the right

thigh looked like fresh meat. The results of the chemical analyses are given on p. 207.

On 27 October, 1916, thirteen and a half months after death the body was very carefully examined, cut up, the parts removed on a barrow and burnt. The skin was very tough, the muscles excellently preserved, except in a part of the area dissected a year previously, where maggots had worked to some extent. The muscles of the shoulder and neck, and lowest part of the body, were softer and more oedematous than those of other regions. The lungs were well preserved, the liver hard, almost normal in appearance, and contained much creosote oil. The stomach and intestines, except the superficial coils on which moulds had grown, were so well preserved that they could be lifted without rupturing. The coecal contents appeared normal in colour, smell and consistency. There was no unpleasant smell, and no fluid oozed out of the body. The whole carcase was so well preserved that it could be dragged by one leg.

Black horse (*B*). By the end of October, 1915, the surface of the exposed meat had become gray-brown and soft, but the change was confined to the superficial layers. Moulds had grown on the intestines and on some of the exposed muscles. Though numerous eggs had been laid near the carcase and on the intestine there were very few living maggots on it. Small dead maggots were very numerous. There was some rancid smell, but insufficient to make very close examination of the body unpleasant. On 7 December little change was noticed, and the carcase could be dragged along by one leg.

On 20 April, 1916, the carcase seemed less shrunken than that of horse (*A*) and had a slight rancid odour. The gluteal muscles where covered by skin were normal in colour, but soft in section and showed small cavities due to the presence of gas. The appearance of the gluteal muscle may be seen in the accompanying photograph (Pl. V, fig. 11). Microscopically some of the fibres were well preserved and showed distinct striae, others were degenerated and stained badly (Pl. V, fig. 13). Degenerated, badly staining bacteria, mostly sporebearers, were found in considerable numbers, especially in the connective tissues.

On 23 May, 1916, portions of meat from (*a*) left shoulder, (*b*) left glutens and (*c*) the upper part of the right thigh were taken for chemical analysis. The meat from the left shoulder was very easy to grind and had a rancid smell, only appreciable on close examination, differing from the smell in the same region of the white horse. The gluteus was

easy to grind and had only a slight smell like that of fresh blood, and the meat from the right thigh was very easy to grind, very moist and had the same smell as (*a*).

On 27 October, 1916, thirteen and a half months after death, the carcase was carefully examined, cut up, and the parts removed in a barrow and burnt. The skin was very tough, and intact in all situations not interfered with by our previous dissections. From the wounds maggots had penetrated during the summer for some distance into the muscular tissues. In situations not affected by the invasion of maggots the muscles and organs had become disintegrated, to a greater or less extent, some parts being moderately well preserved but others being represented by pasty material.

The carcase was dissected and cut into small portions without discomfort from smell or semi-liquid putrid material.

Remarks. In both carcases much intestinal gas developed in the few hours intervening between death and treatment. In the case of the white horse this intestinal gas did not appear to be supplemented by appreciable tissue gas subsequently. Gas, however, was produced in the tissues of the black horse in the first week or two, as it was in smaller skin treated carcases.

The great benefit derived from local supplementary treatment of areas where isolated colonies of maggots developed after two or three weeks was again shown. Both carcases were satisfactory from a practical point of view, but the white horse was distinctly better preserved than the black, especially in the later stages of the long exposure. This agrees with the results obtained with smaller carcases.

Surface treatment is the more easily carried out and the more economical in fluid.

Special attention may be directed to two noteworthy facts; that the skin which had been in contact with the wet ground for many months was tough and well preserved and that the intestinal contents showed little, or no, change.

Chemical analyses.

Specimens from the samples of meat taken on 23 May, 1916, were examined in the following way. Portions weighing about 5 grms. selected for their freedom from connective tissue, were thoroughly ground with 2 grms. of washed sand, triturated with 23·5 c.c. of water and made up to 250 c.c. with 97 % alcohol. After standing 24 hours, the insoluble matter was filtered off on tared papers, dried to constant

weight at 100° C. and weighed. After allowing for the sand the percentage of this dry matter was calculated. The volatile bases and amino acids were then estimated in 100 c.c. of the filtrate by the method previously described (p. 144). The results are given in Table XXXII. We have examined also in the same way pieces of meat taken from the upper shoulder and centre of the upper thigh of an untreated horse on the first, second and third days after death. The incisions made in taking the different samples from the same region did not encroach on one another. The carcase lay during this time in a stable and the weather was very warm and fine. On the third day the carcase was greatly distended, and gas was present in the tissues.

TABLE XXXII.

Analyses of muscles of horses.

Material	Weight of muscle taken	Percentage dry matter insoluble in 96 % alcohol	Volatile bases c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Volatile bases per grm muscle, c.c. N/10 acid neutralised	Formyl titration per grm muscle, c.c. N/10 soda	Ratio of volatile bases to amino acids
White horse							
(*a*) left shoulder	5·122	15·52	5·8	2·3	2·83	1·12	2·52 : 1
(*b*) left gluteus	4·748	23·94	3·3	5·1	1·74	2·69	0·65 : 1
(*c*) right thigh	5·208	19·57	—	—	—	—	—
Black horse							
(*a*) left shoulder	4·789	18·83	13·05	2·45	6·81	1·28	5·33 : 1
(*b*) left gluteus	4·949	19·30	10·4	7·1	5·26	3·6	1·46 : 1
(*c*) right thigh	4·997	13·69	17·45	2·7	8·73	1·35	6·46 : 1
Untreated horse							
(*a*) shoulder 28 hrs.	4·914	24·14	0·6	0·35	0·30	0·18	1·71 : 1
(*b*) thigh 28 ,,	4·886	25·73	0·7	0·45	0·35	0·23	1·55 : 1
(*c*) shoulder 40 ,,	4·794	24·89	0·5	0·45	0·26	0·23	1·11 : 1
(*d*) thigh 40 ,,	5·032	23·36	0·65	0·45	0·32	0·22	1·44 : 1
(*e*) shoulder 62 ,,	5·041	23·84	0·6	0·45	0·29	0·22	1·33 : 1
(*f*) thigh 62 ,,	5·040	24·14	0·7	0·5	0·34	0·24	1·4 : 1

The percentage of dry matter present in tissues taken from various situations in a carcase depends upon the distribution of the fluids as well as upon the amount of disintegration. A low percentage may indicate the presence of fluid or much disintegration. On the other hand a high percentage of dry matter may indicate disintegration after the removal of fluid containing the products, leaving compacted connective tissue, or that little change has occurred. It is obvious therefore that no reliance can be placed upon the percentage of dry matter as an index of the extent of putrefactive changes. For example material from the left shoulder of the white horse contains a low percentage of

dry matter because fluid collected in this situation, and this is correlated with a low ratio of volatile bases to amino acids. The still lower percentage of dry matter in the material taken from the right thigh of the black horse is correlated with the highest ratio. In the case of the black horse the percentage of dry matter in the material taken from the left shoulder and the left gluteus is almost the same, but the ratios show a considerable difference in the amount of putrefactive change.

It will be noticed that considerable differences were found in the results of the analyses of muscles taken from different situations in the two treated horses. In each case the smallest ratio was obtained in the left gluteus where the tissues were driest. In the white horse it is very low, the whole change being probably due to proteolytic activity without the influence of putrefactive organisms. Taking this figure as a standard for this region a little putrefactive change in the black horse is shown (see p. 170). The material taken from the other situations contained much more fluid, and the ratios are higher. Sterile fluid draining from any organ will contain the products of autolysis and hence we would expect to find slightly higher ratios in regions where fluid collects, more particularly if it has come from the abdominal organs where more deamination occurs. The ratio in the shoulder of the white horse is higher than in the gluteus partly for this reason and partly because a little putrefaction had occurred. In the shoulder and thigh of the black horse the ratios are decidedly higher showing that putrefaction had occurred during the long period of exposure.

The differences in the figures obtained by the analyses of materials taken from various parts of a carcase show the importance of a standard disposition of the bodies and of the selection of materials from the same situation, when comparing the rates of putrefaction in muscle or determining the relative effects of disinfectants. The necessity for standard conditions applies also when the fluid exuding from a body is used for analysis.

The results of the analyses of the muscles of the recently killed horse indicate that in three days, in spite of gas formation, no true putrefactive changes had occurred. They show further that little, if any, changes due to the activity of proteolytic ferments had taken place in the muscles. The ratios given in the last columns resemble those found in fresh tissues. The ratios would become lower as proteins were broken down by proteolytic ferments, and subsequently higher as the result of the action of putrefactive bacteria.

Atkinson (1916) reports as follows on an experiment he carried out in Gallipoli on the body of a mule.

"On October 9th the body of a small gray Indian mule, killed by high explosive in the morning, was treated. Holes were made into the thorax and some 'Liquid C' was squirted in. There was a large wound over the abdomen with a portion of colon lying outside it; a counter opening was made and some liquid squirted into the interior, the intestines also being opened by means of a long butcher's knife. The surface of the body, with especial care to all the openings, was sprayed and brushed over with the fluid. In all two gallons were used and 15 minutes taken....

On October 15th the mule was visited again, was slightly distended, but no flies were near or around the body.

October 17th. No flies, and the distension partly subsided.

October 20th. Gas formation was going on rapidly and gas was bubbling from the wound in the intestine. The smell was not great, but was distinct. The mouth was almost half full of the eggs of the blow-fly, and on other patches eggs had been deposited. There was a sloughing patch from an old wound.

October 21st. A further addition of one and a half gallons of 'Liquid C' were used, and some gas from intestine floating in the liquid let out. There was bunch of fly-blow in the mouth which was killed by the fluid.

October 24th. Complete absence of smell and only one small patch outside the nostril where a fly had laid its eggs."

Observations under War Conditions on Human Bodies.

Atkinson (1916) also reports some experiments on human bodies.

"October 9th. The body of a Turk, killed about eight days previously and buried three days, was partially exhumed on the parapet of an old trench. Decomposition was taking place rapidly and the body was covered with a thick swarm of flies. The stench in the area around was very great. The conditions were ideal for trying the usefulness of the liquid under conditions of trench warfare. After about one gallon was sprayed from a short distance over the body, the smell was practically gone. The flies were repelled and the corpse left exposed to the air. The previous night there had been a heavy thunderstorm, so that conditions were favourable for rapid decomposition. It was impossible in day light to spray the whole body as the sniping was too keen.

October 11th. The body was visited again and there was a complete absence of smell and flies. The body was markedly shrunken, and was again sprayed with about a gallon of the liquid. On this day there was again a fall of rain, and three or four other bodies, lying behind the parados between saps 4 and 5, were sprayed also. The smell in their vicinity immediately subsided, and the atmosphere in the trenches adjacent much improved. The bodies had been lying there for over two months.

October 13th. Visited the body of the Turk again after a sharp shower of rain. There was no smell and only an occasional fly settled on the body and immediately went off again.

October 14th. Body gradually shrinking. A few blow-flies were about but did not settle on the body, and there was no smell.

October 18th. After considerable rainfall, no smell or flies.

October 24th. Body shrunken and almost mummified, no smell or flies."

Repelling Flies from Habitations.

In order to ascertain whether the repellent action could be made use of to free dugouts and shelters from flies, several experiments of the following type were tried. A small shelter, about 5 by 5 feet, constructed in a bank, and having a corrugated iron roof, without a window and with a single open doorway, was chosen. This place became very hot on a warm day. Decomposing animal matter (dead rabbits, guinea-pigs, and in one experiment a pig, exhumed after being buried for some days) and fresh excrement were placed inside, a sack hung over the entrance, and the flies, which were soon attracted, were hunted out. The sack was then very roughly sprinkled with creosote oil mixture.

Col. Melville reported on one of these experiments as follows:

"On 12th October, 1915, an imitation dugout was improvised from a shelter on the golf links. This was a small hut, partially turf built and partially excavated, about 6 ft. in height and 5 ft. square. It has a galvanised iron roof and no door. Various animals and other substances in an advanced state of putrefaction were placed inside on 12th October, 1915, and on the 16th when I visited it, the shelter was full of flies and the smell intolerable. The flies were driven out as far as possible, and a piece of sacking hung over the doorway; about half a pint of fluid 'C' was then sprinkled roughly out of a bottle on to the sacking. There were several openings left between the edges of the sacking and the door-posts and lintels of the doorway. We watched the shelter

for 15 minutes, during which period we could see numerous flies trying to effect an entrance, but without success. At the end of the 15 minutes no flies were found in the shelter and there was a complete absence of smell. The shelter was left as it was, the putrid matter being left *in situ*: no more fluid was put on the sacking.

On the 20th October, I revisited the 'dugout.' A few flies had penetrated into the shelter, but these left immediately the curtain was raised; they must have entered through apertures, of which there were several, between the roof and the turf walls of the shelter. There was absolutely no smell in the shelter."

Atkinson (1916) reports as follows: "As a repellent to flies. In this respect the liquid was exceedingly useful; it is best that the beams or wooden structure of messes or dugouts be actually rubbed by the fluid. Spraying leaves patches and the flies seek them out....Such a mess was fly-free for ten days. The other mess at Divisional Headquarters was also sprayed, and was kept free for over six days.

Flies congregate in masses, sometimes two or three deep, in dugouts at night, more especially in stores covered with tarpaulin. The liquid is a most efficient means of dealing with these, as sprayed over them it kills them effectually. Flies that escape with even a small quantity of fluid on their bodies subsequently die.

The tins and seats and immediate surroundings of the latrines belonging to the Divisional Headquarters were sprayed with the liquid, and they were fly-free for four days. About two gallons were used.

Conclusions. The benefit to be derived from liquid 'C' in trench warfare if used in sufficient quantities would be difficult to over estimate. Bodies are mummified by its action and rendered inoffensive, even after and during a fall of rain. It is a very definite fly repellent and will kill the adults in great numbers with quite small quantities from a spray. ...It is the best fly antidote that has been used so far."

It may be pointed out that in Gallipoli the most prevalent flies were *M. domestica*, *F. canicularis*, *F. scalaris*, *C. vomitoria*, *C. erythrocephala*, *L. caesar*, *S. carnaria*, *M. stabulans*, all species which are very common in Europe.

Ross (1916) reported as follows: "Staff Surgeon E. L. Atkinson, R.N., made a very energetic and successful attempt to deal with the putrefying corpses which lay in the open between lines of trenches. These in many cases lay unburied for months owing to the extreme danger a burying party would run, even if working only at night. He supplied me with a fluid, named liquid 'C' for spraying these corpses.

From thirty bodies, which I was able to keep under daily observation, I came to the conclusion, that though the fluid appeared to be non-toxic to flies, they did not congregate on substances sprinkled with it."

In connection with the experiments just quoted Lt.-Col. L. S. Dudgeon has very kindly allowed us to publish the following extracts from a letter he sent to one of us on 30 December, 1915.

"I have only just returned from the Mediterranean area to which I have been attached as a member of the War Office Commission on Epidemic Diseases and Sanitation. I arrived in July and therefore experienced the intense heat and the curse of flies. I was all over ... and in the winter at .... I mention the facts because it will show you that I experienced and saw every possible discomfort and disease produced by flies and dead bodies, both human and animal. All remedies were tried but par excellence in my opinion is solution 'C.' It kills flies at once while distant ones revolve in circles and then join the home only fit for flies. At ... in the advanced line during construction of fresh trenches, the highly offensive bodies of the Turks were removed and if covered with sufficient 'C' work could continue. If no solution 'C' the odour cannot be expressed in words."

THE EFFECTS OF CREOSOTE OIL ON ADULT FLIES.

As we pointed out previously (p. 113) many reagents have a temporary action on adult flies, and it is necessary to watch the insects for a considerable time after treatment in order to ascertain their effects. On many occasions we observed that even very small quantities of creosote oil sprayed on to flies killed them immediately, and that flies only touched with minute droplets soon died. When a putrescent mass of animal matter is sprayed most of the flies caught in the spray die instantly.

Even the vapour of creosote oil is fatal to flies, if they are exposed to it for some hours. In one experiment blow-flies were placed in a balloon trap in a bell jar, one edge of which was lifted so as to allow air to enter. A watch glass containing creosote oil was also placed in the bell jar, some distance from the trap. After two hours all the flies were very feeble and some incapable of movement. All were dead within 18 hours.

Deodorisation.

It has been shown that stinking animal matter may be deodorised by the application of creosote oil. Circumstances may arise when it is impossible to reach such material or when it would be most desirable to treat it from a distance. We have carried out a number of experiments to ascertain how far spraying is effective for such a purpose. The fluid is capable of application through the finest sprayers and small quantities applied by this means will very rapidly deodorise putrefying materials, so that they can be approached without discomfort and more thoroughly treated. Bodies which have been sprayed become offensive, if disturbed, but if undisturbed attract few flies and only become offensive gradually.

Professor I. Walker Hall wrote to one of us as follows: "This solution has been of great use in removing the smell from putrefying tissues in both animal and human bodies. When sprayed on rats, which had been found dead and sent for examination in an advanced stage of decomposition, the nauseous smell disappears almost immediately.

In two post-mortem examinations made during August, 1916, upon persons who had died six days previously, the smell was so offensive that it could be detected 50 yards away, although the bodies were shut up in a room. In each instance the cadaver was almost covered with flies. Before we had time to spray completely the surface of the body and the internal organs, the offensiveness had disappeared and the autopsy was carried out without any discomfort or repulsion.

When making post-mortem examinations upon rats suspected of plague this solution was used to protect the workers from the bites of rat fleas. It was sprayed over the whole skin of the animal. If fleas or lice were present they came out from the deeper parts of the hairs and appeared on the surface. They did not make any attempt to jump and seemed stupefied. A 50 % solution in alcohol served equally well."

Maggots in Manure.

The large heap of manure already infested with larvae presents a difficult problem. Many points such as the kind, age, method of storage, the extent and kind of fermentation in relation to the food supply of the larvae and their distribution and migratory habits have to be considered. Some of our preliminary observations show that

the distribution of the larvae depends upon the temperature, which depends upon the nature and extent of the fermentation and this in its turn upon the air supply, and ultimately the air supply depends upon the wetness and compactness of the heap. Horse faeces contain much less water than cattle faeces, and horse manure is termed "hot" because the large amount of air in it permits of the growth of aerobic organisms, which are responsible for the oxidation of carbohydrate material and the consequent increase in temperature. If manure is sufficiently wetted and compacted diffusion of air into the heap is prevented, the air remaining in it is soon used up, the carbon dioxide produced by the aerobes saturates the heap, and anaerobic conditions, which are not accompanied by any considerable rise in temperature, prevail.

We have found the superficial and deep distribution of maggots very irregular in heaps composed of the manure of different animals, and especially so in manure mixed with offal.

The attempt to destroy maggots in large heaps of farmyard manure is likely to be attended with considerable difficulty as water soluble larvicides must be employed to insure sufficient penetration to reach all the maggots, if the temperature is not high. We have already pointed out the resistance of maggots to extremely toxic larvicides, when these are applied in the form of solutions (p. 186). It is therefore evident that the quantity of soluble substances it is necessary to add to reach the necessary concentration throughout the bulk of the manure prevents their effective use from the economical standpoint. The possibility of the fixation of the potent constituents by the organic matter must also be borne in mind.

Fluid larvicides, insoluble or only slightly soluble in water, including those of an oily character, when applied to the surface would not diffuse sufficiently into the mass.

The successful treatment of manure should result in the prevention or destruction of larvae without injury to the manure for agricultural purposes. The difficulties attending the successful treatment of heaps already infested with larvae are evidently so considerable that we sought for other means of solving the problem, and concluded that the easiest method would be to treat the manure at the earliest possible moment, before the heaps were constructed. Our experience with creosote oil indicated that this would be a suitable reagent to try, and we are indebted to Mr J. E. M. Mellor for carrying out some experiments on the lines we suggested to him.

It has been shown that flies are only attracted to human faeces for three or four days (Graham-Smith, 1916, p. 492) and Mr Mellor's observations indicate that for the purpose of depositing eggs house flies are attracted mainly to fresh horse manure. His experiments, which will be published shortly, show clearly that the superficial treatment of a manure heap, already infested with fly larvae, with creosote oil at the rate of 4 gallons to the ton, is of little value, since large numbers of flies eventually emerge. On the other hand he has shown that if fresh horse manure exposed for 24 hours is sprayed "incrementally" at the rate of one gallon to the ton the results are satisfactory. The smell which attracts the flies is diminished to a large extent, most of the eggs already deposited are killed, a large proportion of the maggots which do hatch die, the creosote oil is distributed throughout the heap subsequently made, flies are repelled from it, and few emerge. Such "incremental" treatment can be easily and cheaply carried out, is more effective than any attempt to treat maggot infested heaps, and so far as we have been able to ascertain does not apparently have any injurious effect on the manure[1]. Further experiments are necessary before we can determine the minimal quantities that should be used, but we believe the method is worthy of trial on a large scale.

The Prevention of Flies in Towns.

In towns flies deposit their eggs in stable manure, dust bins, middens, refuse heaps, and similar situations, and the larvae develop in collections of horse manure and refuse tips. If the method we have advocated for dealing with manure by "incremental" spraying is found to be effective, the same means could be made use of in dealing with the breeding places of flies in towns. The fresh manure in stables should be sprayed daily thus eliminating the odour attractive to flies, killing the eggs already deposited and rendering the heaps unsuitable for the larvae. We believe that in the absence of large quantities of straw, spraying at the rate of 100 c.c. per horse per day, when the manure is collected, would suffice. The cost per ton of manure would be very small. Since maggots pupate at the margins of the yards these might be treated with advantage. Dust bins used for house refuse are very attractive to flies of many species which deposit their eggs in them. The film of semi-liquid putrescent material, which invariably covers

[1] Applied "incrementally" at the rate of one gallon to the ton the treatment does not interfere with processes responsible for the increase in temperature.

the bottom and sides of the bins, is never removed, causes the bins to be attractive at all times and affords food material to the maggots. On each occasion when the bin is emptied a small quantity of creosote oil should be poured into it. This procedure would tend to disinfect the film, kill any eggs or maggots present in it, eliminate the odour attractive to flies, and prevent them from visiting the bins. If the owners could be induced to spray the contents daily with a sprayer delivering about 10–20 c.c. these very prolific breeding grounds would no longer be a source of nuisances from smell or flies. The expense would be negligible, since a gallon would suffice for the fly season from the beginning of April to the end of October.

If dust carts were provided with suitable sprayers of one gallon capacity so arranged that the contents of the cart could be sprayed at frequent intervals through the cover by turning a handle and thus distributing the gallon throughout the load, an additional safeguard would be provided against the development of eggs and larvae present in the material from neglected bins. The refuse tips would require no further treatment.

There should be no difficulty in devising suitable means for treating privies, earth closets, middens, etc., and preventing nuisances, when they were being cleaned out.

Some Objections to the Use of Creosote Oil Mixtures.

Atkinson (1916) has summarised the main objections to the use of creosote oil in the following words. "The liquid is an irritant and it is well to wash the hands after use. It burns the face slightly, if left on. Protective glasses should be worn by men using the spray....The liquid is extremely inflammable and very great care must be taken not to have a naked flame near when spraying dugouts and messes."

It is true that the liquid has a transitory irritant effect on the mucous membranes, especially of the eye, but we have never experienced irritant effects on the skin, though working with it for hours at a time with it on our hands. The fluid can be removed very easily from the skin with a little spirit. Nevertheless it would be best for those who might have to work much with such fluids to protect the eyes with glasses, when using a spray in windy situations.

The flash point in an "open cup" is 193–194° F. In an apparatus which gives results corresponding closely to those obtained by the Abel tester the flash point is higher, namely 199–200° F. This flash point

is more than two and a half times that allowed in the transport of dangerous fluids, and we are of opinion that the danger is negligible with ordinary methods of transit. In the form of a very fine spray the liquid is inflammable, and the precautions suggested by Atkinson should be taken.

The colour, taste and smell of the liquid are such that no precautions need be taken to prevent its being swallowed.

It has been suggested to us that, since dilution is undesirable, difficulties in transport render the extensive use of such liquids costly. We have shown that the most important factor in the preservation of a carcase is the treatment of the skin in such a manner as to prevent the ingress of maggots, putrefactive organisms, water, and perhaps air into the tissues. This can be most easily done by applying an oily material containing disinfectants, slightly soluble in water, by means of a brush so as to produce a continuous film. No 5 % emulsion, prepared by means of soft soap or otherwise, or solution can accomplish this purpose, because the water of the emulsion softens the skin and a continuous film is not produced. The putrefactive bacteria protected by greasy constituents in the follicles, etc., are neither destroyed nor imprisoned as the skin is not hardened. The disinfectants are more liable to fixation and removal by dilution and letching. The deficient distribution and concentration would not prevent smells and maggots from developing in a short time, necessitating very frequent applications with comparatively poor results. In our opinion any economy in transport by the use of 5 % emulsions is far outweighed by the great economy in labour and incomparably better results obtained by the use of undiluted oily disinfectants.

For many purposes a spray of a suitable fluid may be used, for example for destroying eggs and maggots working superficially, for deodorising putrescent material, for repelling flies from habitations, putrefying substances, faecal material, latrines, dust bins, etc., treating infected soil, or fresh manure containing fly eggs. A fluid suitable for such a purpose should be capable of use with the finest sprayer, adhere to greasy surfaces, spread and tend to form a film over comparatively large areas, retain its properties for a long time, not be washed away by rain, or have the concentration of its potent ingredients rapidly affected by extraction with water. Watery emulsions are incapable of exercising most of these functions.

Instructions for using Creosote Oil Mixtures.

Our experience has shown that creosote oil mixtures may be used with very great advantage for a variety of purposes, and we therefore indicate briefly the most satisfactory methods of applying them for different purposes. These methods are so simple that no difficulties should arise in their practical application.

(*a*) A carcase that cannot be disposed of immediately should be sprinkled over with creosote oil mixture by means of a watering can, and the fluid distributed in the direction of the hair with a hard brush. When one side has been treated, including the extremities, the carcase should be turned over and the other side treated in the same way. The abdominal and thoracic cavities may be opened and some of the fluid poured into them, but this does not appear to be necessary. If the abdominal cavity is opened it may be desirable to puncture the gut in order to allow the gases to escape, and the fluid to find its way amongst the coils. By means of a funnel some of the fluid should be poured into the gut through the punctures. Then small quantities should be poured into the mouth, eyes, ears, anus and any wounds there may be. In this climate about a gallon suffices for treating a horse, half a gallon for the external treatment and the rest into the serous cavities, if they are opened. Two men can easily treat a horse in 15 minutes. While the gases from the intestines are escaping there may be some smell but this phase soon passes off, and the very disagreeable odours due to putrefaction do not arise. This treatment will preserve the carcase satisfactorily for some weeks, keep off flies for some days and prevent the deposition of their eggs, or, if eggs are present, the hatching of the larvae. If fly larvae are present before the carcase is treated they will be killed. If the carcase cannot be disposed of within two or three weeks, it may be necessary to make use of small local supplementary applications.

(*b*) As the method described above has proved satisfactory, we do not now advocate under ordinary conditions the process of injection. Injection through the carotid artery is, however, easily accomplished, and a body thus treated in addition to the skin treatment is preserved for months.

(*c*) Putrefying bodies should be sprayed from a distance. Almost immediately the stench will be much diminished, if not entirely obliterated. If the body can be reached, it should be treated thoroughly. Other foul-smelling materials can be treated in the same way.

(*d*) Fly larvae can be destroyed by spraying or preferably sprinkling the fluid over and into the spaces in infested materials. In a short time all the maggots will be dead. For the treatment of manure, dust bins, latrines, etc., see p. 216. All flies touched by the spray will be killed.

(*e*) All flies should be as far as possible driven out of shelters or rooms and pieces of cloth sprinkled with creosote oil mixture hung over the places where they can enter. By this means the majority of flies can be prevented from entering for some days.

(*f*) Such fluids should not be emulsified with water.

(*g*) For disinfecting liquids an alcoholic solution of the fluid can be used, which on addition to water makes a milky opalescence throughout the fluid owing to the separation of the oil in the form of extremely minute globules.

Conclusions.

1. Experiments with the bodies of horses show that they behave in the same manner as those of smaller animals, and can be efficiently treated in the same ways. A body was preserved by surface treatment alone for a long period, and produced no appreciable nuisance from smell.

For large carcases relatively less fluid is required for surface treatment owing to the relatively small ratio of surface to weight.

2. The treatment of human bodies under war conditions has given satisfactory results.

3. Flies have been kept from entering such places as dugouts by hanging sacks treated with creosote oil mixtures over the entrance.

4. Adult flies in dugouts and other situations and on putrescent material are killed by spraying.

5. Latrines have been kept free from flies by spraying.

6. Manure should be treated by spraying with creosote oil at the earliest opportunity. If made into heaps each incremental addition should be spread uniformly on the heap and sprayed at the rate of at least 100 c.c. per horse per day. The manure does not seem to be injured by this treatment

7. In towns the breeding places of flies could probably be treated with little expense, and the numbers of flies very greatly diminished.

8. The chief objections to the use of creosote oil for such purposes are (*a*) its irritant action on the skin and mucous membranes, (*b*) its inflammability and (*c*) difficulties in transport. In view of the excellent results obtained the objections are of little importance. In our

experience its irritant action on the skin is very slight, and the eyes can be protected by the use of glasses when spraying; its inflammability is low except when used as a spray, and suitable precautions could be easily employed. The difficulty and cost of transport have to be weighed against the economy in labour, since a single treatment with creosote oil is more efficient than many with 5 % emulsions of disinfectants.

General Summary.

1. Flies may be killed either by poisons (*a*) absorbed from the alimentary tract, or (*b*) acting through the respiratory system. They are very resistant to many alimentary poisons which possess considerable toxicity to animals, but are more susceptible to respiratory poisons.

2. As very little difference could be made to the general fly population by killing adults alone we have not persisted with experiments designed for this purpose. Aniline is the most suitable of the reagents, not dangerous to man, used in the way suggested, which we have tested.

3. Flies are most easily and effectually destroyed by attacking them in their immature stages as eggs or larvae.

4. The eggs of species likely to be dangerous to man by conveying infected material to his food are laid on (*a*) exposed animal matter, (*b*) manure, and (*c*) refuse.

The eggs and maggots in these situations may be considered to represent large numbers of flies in traps.

5. For killing eggs or larvae in their breeding grounds we have found coal-tar oils, especially creosote oil, to be the most satisfactory reagents. Aniline emulsions are useful, but have little effect on putrefactive processes and the nuisances due to them.

6. Flies may be repelled from substances which attract them, such as decaying bodies, faecal material, etc., and kept out of habitations by means of the repellent constituents of coal-tar oils.

7. Flies sprayed with these oils are killed.

8. In carcases true putrefaction or disintegration is preceded by (*a*) early gas formation, mainly due to the action of intestinal organisms on the carbohydrates of the intestinal contents and tissues, (*b*) exudation of fluid, probably due to the effects of cytolysis and enzyme action, and (*c*) green discoloration of the skin which appears to be connected with the effect of hydrogen sulphide or organic acids on the blood pigments. By suitable treatment the tissues may be rendered sterile, when neither gas nor green discoloration is produced though fluid exudes.

9. By true putrefaction in carcases we mean the breakdown of the tissue constituents, accompanied by the elimination of foul-smelling products. The process is due to the activity of putrefactive bacteria assisted by the action of tissue enzymes. Gas production and exudation of fluid continue as true putrefaction proceeds, but in much smaller daily increments than in the preliminary stages.

10. Descriptive methods are lacking in precision and do not give definite information regarding the progress of putrefaction. The need arose therefore for a method by which the actual products of putrefaction could be estimated. The importance of the combined activity of autolytic enzymes and putrefactive organisms in the disintegration of a carcase was impressed upon us by noting the great rate of production of volatile bases in tryptic digests containing such bacteria.

The putrefactive powers of various species of bacteria can be measured definitely by incubating an amino acid mixture containing the organisms under standard conditions for a suitable time and determining the ratio of bases to amino acids.

We claim that by similar means the relative powers of different disinfectants to inhibit the action of putrefactive organisms on carcases (kept under standard conditions, p. 209), can be compared precisely, using for analysis the fluids which exude or tissues from comparable situations.

The proteolytic as well as the deaminating enzymes of autolysis produce small amounts of ammonia. The results of their combined activity, in the absence of organisms, yield a low ratio of volatile bases to the substances which respond to the formyl titration. If putrefactive organisms do not develop in a treated carcase the same low ratio is obtained. The ratio is correspondingly greater the more active the organisms.

Our method enables us to measure the progress of putrefaction under all conditions, provided the reagents used to inhibit putrefaction do not interfere in the estimations.

11. The stenches arising during putrefaction are mostly derived from acid and basic products and from sulphur bodies. An ideal deodorant should be capable of fixing or absorbing all foul-smelling bodies.

12. We believe that putrefactive bacteria mainly gain entrance into the tissues through the skin.

13. The presence of water and a high temperature provide optimum conditions for the progress of putrefactive changes.

14. In the superficial treatment of intact or opened carcases and other putrescible materials reagents should be used which adhere to

the greasy surfaces, form films, render the skin waterproof and kill the bacteria in it, thus checking putrefaction by preventing the access of water and putrefactive bacteria to the tissues. Further the reagent should be capable of eliminating any stenches which may arise, repelling flies, killing the eggs or larvae, resisting the action of water and remaining operative in all respects for a long period.

15. Watery emulsions of disinfectants are necessarily deficient in most of these properties. Undiluted oily reagents only possess them.

16. By superficial treatment combined with injection of certain reagents into the blood vessels exposed carcases may be preserved for months.

17. The burial of carcases does not prevent the development of larvae present on them, or the subsequent emergence of the flies.

18. In our experience the reagent, which possesses the required properties to the greatest extent, and gives the most satisfactory results in practice and is sufficiently cheap and easily obtained for use on a large scale, is coal-tar creosote oil of "country make."

19. For general purposes, especially when the repelling of flies is of importance, we recommend the use of coal-tar creosote oil of country make, containing a high percentage of phenolic bodies, to which sufficient bases, extracted from "light oil," are added to make the proportion of bases to phenolic bodies approximately one to two.

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DESCRIPTION OF PLATES I—V.

PLATE I.

Fig. 1. The bodies of the two horses, *A* above and *B* below, on 6 October, 1915, 29 days after death. The wounds made in the thorax of the black horse, *B*, in order to introduce the fluid into the thorax are visible. Some of the gut, which was pulled out of the abdominal wound, is seen lying on the abdomen.

PLATE II.

Fig. 2. The body of the white horse, *A*, on 14 Sept. 1915, seven days after death. The abdomen is somewhat distended with gas, and the injection wound in the neck is visible.

Fig. 3. The body of the white horse, *A*, on 4 October, 1915, 27 days after death. The abdomen is less distended.

Fig. 4. The body of the white horse, *A*, partly dissected, on 6 October, 1915. The gloss on the peritoneal surfaces of the intestines and the excellent condition of the abdominal wall is well seen.

PLATE III.

Fig. 5. Dissection of the superficial muscles of the gluteal region and leg of the white horse, *A*, on 6 October, 1915. The reflected skin is seen lying below.

Fig. 6. Dissection of the deeper muscles of the gluteal region of the white horse, *A*, on 6 October, 1915. The normal appearance of the hair should be noted.

PLATE IV.

Fig. 7. Further dissection of the white horse, *A*, showing the normal appearance of the abdominal organs.

Fig. 8. Dissection of the black horse, *B*, on 6 October, 1915, showing part of the intestines with glossy peritoneal surfaces, and a deep incision into the muscles of the hind quarters. The skin has been reflected downwards, and a large mass of muscle is hanging down behind the reflected skin. A portion of the gut, which had been exposed throughout on the surface of the abdomen, is seen at the upper margin of the abdominal wound. (Cf. Fig. 1.)

Fig. 9. The body of a skinned guinea pig 24 hours after treatment with creosote oil showing great numbers of large maggots dead and black.

PLATE V.

Fig. 10. Vertical section ([illegible]) through the skin and left gluteal muscle of the white horse, *A*, made on 20 April, 1916, seven and a half months after death.

Fig. 11. Vertical section ([illegible]) through the skin and left gluteal muscle of the black horse, *B*, made seven and a half months after death. Small gas bubbles are visible throughout the section.

Fig. 12. Section of muscle taken on 20 April, 1916, seven and a half months after death, from the left gluteus of the white horse, *A*. The fibres are regular and the striae very evident.

Fig. 13. Section of muscle taken on 20 April, 1916, from the left gluteus of the black horse, *B*. The fibres are irregular, and some are degenerated. The striae are irregularly dispersed but are well marked in some of the fibres.

Fig. 14. Photograph of rabbit (*D*) after removing the skin of the right side on the 19th day after death. The body was injected with creosote oil (9 c.c. per lb.) and the skin treated with 15 c.c. per lb., and it was allowed to lie on the ground without protection.

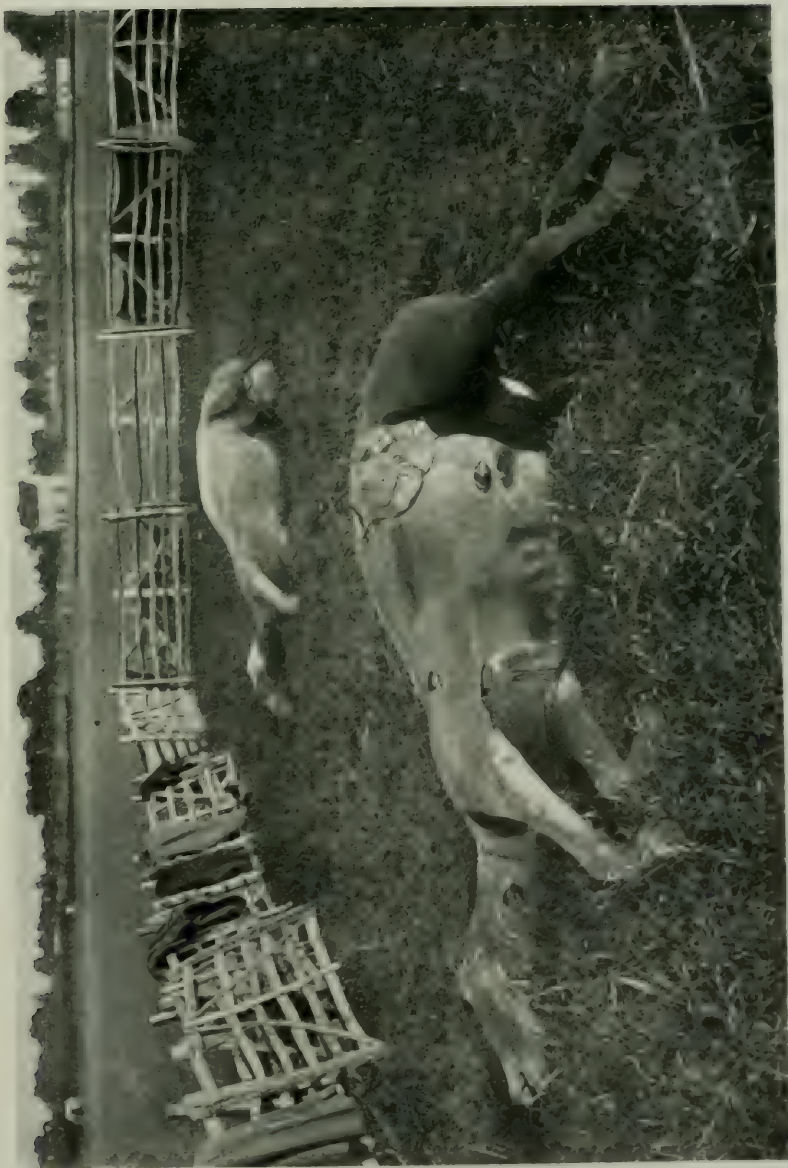

Fig. 1

Fig. 2

Fig. 3

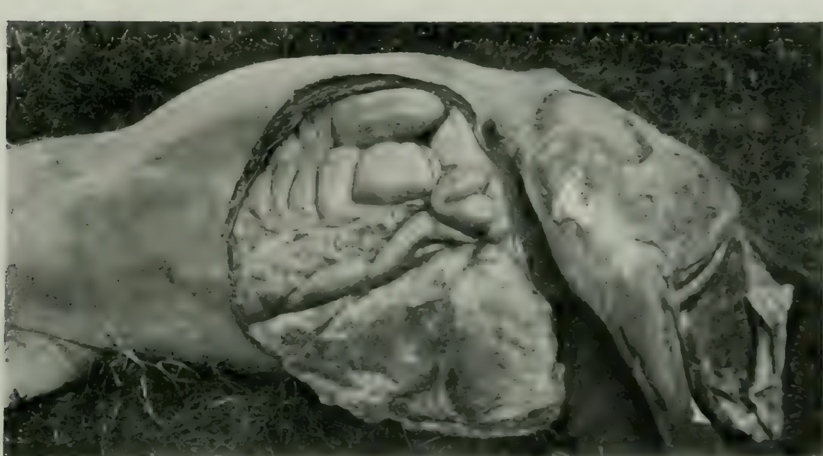

Fig. 4

Fig. 5

Fig. 6

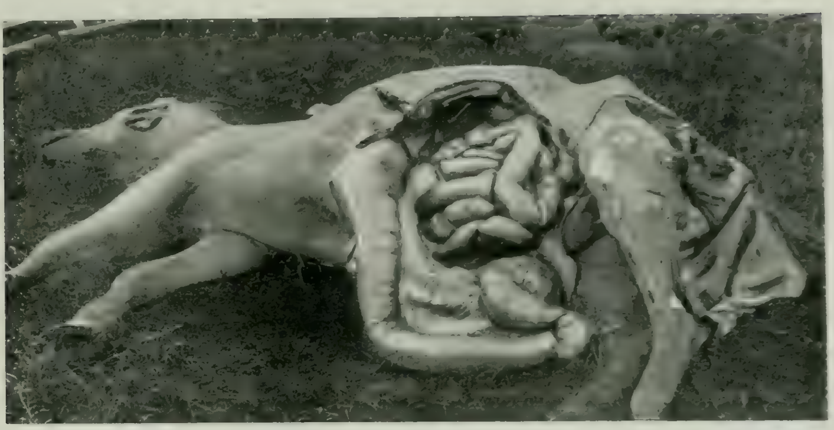

Fig. 7

Fig. 8

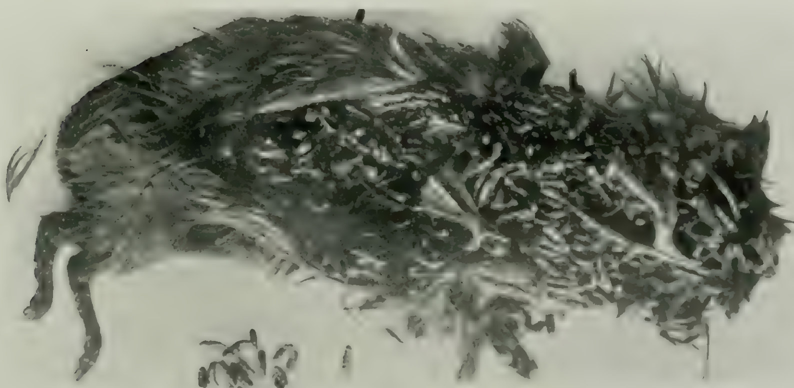

Fig. 9

Fig. 10

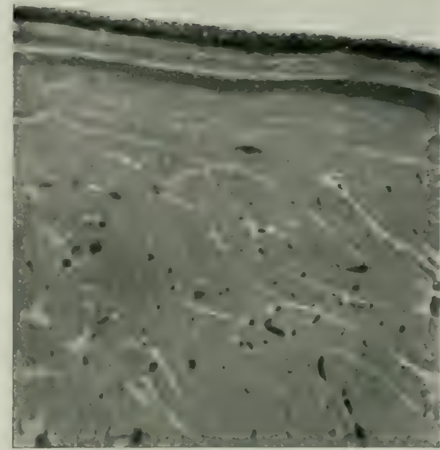

Fig. 11

Fig. 12

Fig. 13

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THE VITALITY AND VIABILITY OF STREPTOCOCCI IN WATER.

BY WILLIAM G. SAVAGE, M.D., B.Sc. (LOND.)
AND D. R. WOOD, F.I.C.

(*From the Somerset County Public Health Laboratory.*)

(With 4 Text-figures.)

IN an earlier paper from this Laboratory the significance of streptococci in water supplies was dealt with from the point of view of the comparative occurrence of streptococci and *B. coli* in different classes of waters. It was demonstrated that these two groups of organisms corresponded fairly closely as regards their numerical presence in water supplies. It was pointed out that "while we do not know enough about the varying vitality and distribution of streptococci to say whether the presence of certain strains may or may not be disregarded as evidence of excretal contamination it is, in general, reliable to assume that streptococci in large numbers are only present in waters from unsatisfactory sources."

The bacteriologist who has to report upon water supplies is always seeking for data, which as yet he does not possess, which shall give him information upon two points whether the excretal pollution detected is animal or human and as to the date and recency of any contamination. The series of experiments recorded here were designed to throw light upon the second point and more particularly from the point of view of streptococci in water.

While Houston, Horrocks and others have carried out investigations upon the viability of isolated streptococci in water or sewage and Prescott and Baker have studied the relative growth of *B. coli* and sewage streptococci from polluted waters in glucose broth we are unaware of any work dealing with the comparative vitality of *B. coli* and streptococci in water under nearly natural conditions.

It seemed to us that experiments upon these lines would supply data of value in determining the relative significance of these organisms in water.

Methods of investigation.

To study the comparative viability of streptococci and *B. coli* in water cylindrical earthenware tanks were used each of a diameter of 12 inches and a capacity of about 50 litres. They were obtained by using large drainpipes with a cement bottom but open at the top. These tanks were kept in the open air, lightly covered to prevent rain or dust access but with free air admission. The temperature of the water was recorded daily. About 40 litres of unsterilised tap-water (a bacteriologically good water) was put into each tank to which was added varying quantities of sewage or excreta. Preliminary experiments with samples of sewage and excreta showed great variations in their *B. coli* and streptococcus content so that it was not possible to add always the same amount. This however was quite immaterial, the aim being to have about 1000 *B. coli* or streptococci per c.c. of the final mixture without making it a nutritive material. In view of the comparatively fewer streptococci in animal excreta it was found better in the later experiments to first mix a definite amount of the excreta with a little sterile water, allow the coarser particles to separate and then add the bacterially rich upper emulsion to the tank water. In this way the majority of the bacteria were added with comparatively little organic matter.

After the addition of the excrementitious material the mixture was very thoroughly stirred and the first sample collected. In the first three recorded experiments (I, III, IV) the water was stirred before each sample was taken. In the other experiments no further stirring was resorted to until the end of 4 weeks. In a number of cases duplicate samples were collected one just before, the other just after, stirring, to study how far any diminution was due to sedimentation without loss of viability.

Contrary to our expectations very little difference was made by stirring the water at the end of 4 weeks. In Exps. IX, X, XI, or XV, practically no increase in either organism was observed. In Exp. XVI some slight increase in the number of streptococci was recorded, while in Exp. XIII there was a considerable increase in the number of *B. coli*, but in this experiment there was an appreciable amount of deposit. Our results show that, in the absence of sediment, these bacteria do not appreciably precipitate and remain alive, even in perfectly still water.

In a few of the experiments when one type persisted long after the other had died out, at the end of 4 weeks or longer (but never earlier) the completion of the experiment was studied by transferring part of the water to a large sterile bottle kept under similar conditions.

To examine the tank water samples were collected in sterile bottles and examined immediately.

The *B. coli* enumerations were made by the usual lactose bile salt broth method using lactose bile salt neutral red agar for plating out. The streptococcus enumerations were made by the method we have described elsewhere, i.e. by adding definite fractions of the water to neutral red broth (single or double strength) and examining for streptococci in hanging drop preparations after incubation for 40–48 hours at 37° C. Only cocci in quite definite chains were taken as evidence of the presence of streptococci while negative results were only recorded after repeated examinations. In doubtful cases the deposit was centrifugalised and stained.

To avoid wide spacing of results amounts intermediate to the usual decimal dilutions were also employed, *i.e.* 0·03, 0·3, etc. as well as 0·01, 0·1, 1·0. Such intermediate amounts approximate closely to the mean in geometrical progression between 10 and 1 c.c., 1 and 0·1 c.c., etc. (viz. 3·16, 0·316, etc.), and the quantities taken for examination are thus uniformly spaced, each quantity being approximately one-third the quantity preceding it in the series.

The experiments upon the viability of individual strains of streptococci and *B. coli* were carried out in stoppered bottles containing one litre of tap-water, kept in diffuse daylight at room temperatures, a cool North room being utilised. The tap-water used was a very hard one so, in order to avoid mineral sediment, the water was first boiled and the precipitated calcium carbonate filtered off before the sterilisation.

We desire in particular to point out that the amounts of excrementitious material added were in no case sufficient to convert the water into a nutrient material. This is a matter of considerable importance as we wished our experiments to be under strictly practical conditions. The amounts added were not more than might readily occur under natural conditions of rather gross pollution. Free and albuminoid ammonia determinations were carried out in a number of cases at the start of the experiment and they showed that the organic matter added was not considerable. For example:

Exp. XV.	Cow excreta and water	Free = 0·004	Albuminoid = 0·032
Exp. XVI.	Sewage excreta and water	,, = 0·008	,, = 0·007
Exp. XXII.	Human excreta and water	,, = 0·001	,, = 0·008
Exp. XXIII.	Cow ,, ,,	,, = 0·026	,, = 0·085

Exps. II, XII and XIV did not give satisfactory relative numbers of the two organisms so were not followed out and are not recorded.

Details of the experiments.

Group A. Comparative viability in water of B. coli and streptococci derived from human excreta.

Four experiments were carried out. The actual figures of the numbers present per c.c. are set out in Table I and the percentage survival in Table II.

TABLE I. (*Numbers per c.c. of the water.*)

Examination intervals	Exp. IX		Exp. X		Exp. XI		Exp. XXI	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	1000–3000	30–100	100–300	100–300	1000–3000	100–300	30–100	1000–3000
3 days	100–300	30–100	300–1000	300–1000	1000–3000	30–100	10–30	30–100
7 „	10–30	3–10	10–30	1–3	300–1000	10–30	3–10	100–300
2 weeks	10–30	absent*	0·03	absent	3–10	0·1	1–3	1–3
3 „	1–3	„	0·03	„	3–10	0·03	0·03	1–3
4 „	0·3	„	0·03	„	3–10	absent	absent	0·03
4 „ (after stirring)	1–3	„	0·03 (3 weeks)	0·03 (3 weeks)	3–10	0·03	—	—
5 weeks	0·1	—	absent	absent	0·3	0·03	absent	absent
6 „	0·03	—	—	—	0·1	absent	—	—
7 „	absent*	—	—	—	absent	„	—	—

* *absent* = absent from 30 c.c.

Exp. IX. 0·5 grm. normal human excreta to 40 litres tap-water. Sept.–Nov. average temperature 52° F.

Exp. X. Excreta from convalescent case of paratyphoid fever. Emulsion of 4 grm. in 100 c.c. sterile water. After a few minutes settlement two-thirds of supernatant fluid added to 40 litres tap-water. Nov. and Dec. average temperature 40·1° F.

Exp. XI. Two grm. excreta from another convalescent paratyphoid fever case to about 40 litres tap-water. Nov., Dec., Jan. average temperature 37° F.

Exp. XXI. 3·0 grm. excreta from a suspected typhoid bacillus carrier (no typhoid bacilli found) added to 40 litres tap-water. March and April average temperature 42° F.

TABLE II. *Percentage survival.*

Examination intervals	Exp. IX		Exp. X		Exp. XI		Exp. XXI	
	B. coli	Strepto cocci	*B. coli*	Strepto cocci	*B. coli*	Strepto cocci	*B. coli*	Strepto cocci
Start	100	100	100	100	100	100	100	100
3 days	10	100	300	300	100	30	33	3
7 „	1	10	10	1	30	10	10	10
2 weeks	1	0	0·03	0	0·3	0·1	3	0·1
3 „	0·1	0	0·03	0	0·3	0·03	0·1	0·1
4 „	0·03	0	0·03	0	0·3	0	0	0·003
4 „ (after stirring)	0·1	0	0·03	0·03	0·3	0·03	—	—
5 weeks	0·01	—	0	0	0·03	0·03	0	0
6 „	0·003	—	—	—	0·01	0	—	—
7 „	0	—	—	—	0	0	—	—

Remarks. All four experiments show a rapid diminution in the number of both groups of organisms which is so marked that at the end of as short a period as two weeks they are either absent or present in

insignificant numbers only (except in one experiment). This elimination does not markedly favour one group more than the other, the chief difference being that the final disappearance of *B. coli*, as shown by its absence from 30 c.c., is usually much more protracted than for streptococci.

Group B. Comparative viability in water of B. coli and streptococci derived from animal excreta.

Three experiments, the results being shown in Tables III and IV.

TABLE III.

Examination intervals	Exp. IV *B. coli*	Exp. IV Streptococci	Exp. XIII *B. coli*	Exp. XIII Streptococci	Exp. XV *B. coli*	Exp. XV Streptococci
Start	1000–3000	1–3	100–300	100–300	30–100	100–1000
3 days	—	—	30–100	0·3	1–3	3–10
7 ,,	300–1000	0·03	30–100	0·3	3–10	10–30
2 weeks	100–300	absent	10–30	0·1	1–3	30–100
3 ,,	30–100	,,	absent	absent	0·1	0·03
4 ,,	3–10	,,	,,	,,	0·03	0·03
4 ,, (after stirring)	—	—	1–3	,,	—	—
5 weeks	1–3	absent	0·3	,,	0·03	absent
6 ,,	0·3*	—	0·03	—	0·03	,,
7 ,,	—	—	absent	—	absent	—

* This organism survived for long periods. It was found present in 10 c.c. of the water at the end of 11 and 16 weeks respectively. In its cultural characters it fermented lactose only slightly but in other respects was typical producing indol, clotting milk, etc. It showed capsule formation.

Exp. IV. 2·0 grm. sheep excreta to 41 litres tap-water. May to Sept. 1916. Average temperature 61° F.

Exp. XIII. 30 grm. of cow excreta emulsified with water and strained through muslin. The filtrate added to about 40 litres tap-water. Dec. 1916 and Jan. 1917. Average temperature 48° F.

Exp. XV. 30 grm. of cow excreta emulsified with water and the filtrate after straining through muslin added to about 40 litres tap-water. Jan. and Feb. 1917. Average temperature 33° F.

TABLE IV. *Percentage survival.*

Examination intervals	Exp. IV *B. coli*	Exp. IV Streptococci	Exp. XIII *B. coli*	Exp. XIII Streptococci	Exp. XV *B. coli*	Exp. XV Streptococci
Start	100	100	100	100	100	100
3 days	—	—	30	0·3	3	3
7 ,,	30	3	30	0·3	10	10
2 weeks	10	0	10	0·1	3	30
3 ,,	3	0	0	0	0·3	0·03
4 ,,	0·3	0	0	0	0·1	0·03
4 ,, (after stirring)	—	—	1	0	—	—
5 ,,	0·1	—	0·3	—	0·1	0
6 ,,	0·03	—	0·03	—	0·1	0
7 ,,	—	—	0	—	0	—

Remarks. As for Group A there is a rapid diminution of both *B. coli* and streptococci but with a more prolonged mere persistence of *B. coli* in small numbers. The survival was rather longer than with human excreta, the results after 3 weeks being more nearly comparable to those with human excreta after 2 weeks.

It is interesting to note that the surviving *B. coli* in both Exp. XIII and XV was of the type described by Clark and Lubbs, having its origin in grain, and distinguishable from the normal excretal type of *B. coli* by the low final concentration of hydrogen ions produced in glucose hydrogen di-potassium phosphate peptone water medium. They are called by these authors "high ratio" organisms since the ratio $CO_2 : H$ produced in glucose media exceeds that of normal *B. coli*.

Group C. Comparative viability in water of B. coli and streptococci derived from sewage.

Four experiments, the results being shown in Tables V and VI.

TABLE V.

Examination intervals	Exp. I		Exp. III		Exp. XVI		Exp. XX	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	100–300	10–30	30–100	30–100	100–300	100–300	300–1000	300–1000
3 days	30–100	3–10	100–300	30–100	over 1000	300–1000	100–300	300–1000
7 ,,	0·3	0·03	3–10	0·3	100–300	300–1000	10–30	100–300
2 weeks	0·1	0·03	0·1	0·1	30–100	3–10	3–10	1–3
3 ,,	0·03	0·03	0·1	0·1	10–30	3–10	0·3	0·03
4 ,,	0·03	0·03	0·03	0	1–3	3–10	0·3	0·03
4 ,, (after starting)	—	—	0	0	—	—	—	—
5 weeks	0	0·03	0	0	0·3	10–30	1–3	0·03
6 ,,	0	0	—	—	0·1	3–10	0·03	0
7 ,,	0	0	—	—	0·03	0·03	0	0
8 ,,	—	—	—	—	0	0·1	—	—
9 ,,	—	—	—	—	—	—	—	—

Exp. I. 165 c.c. domestic raw sewage added to 41 litres tap-water. Feb., March, April, 1916. Average temperature 38° F.

Exp. III. 100 c.c. domestic raw sewage to 41 litres tap water. April and May, 1916. Average temperature 53° F.

Exp. XVI. 200 c.c. of a mixture of several sewage samples added to 40 litres of tap water. Jan., Feb., March, 1917. During part of this period the tank water was frozen hard and only a little water could be obtained for sampling. The tank was cracked by the frost but several litres of the water were obtained and transferred to a sterile bottle and the experiment continued in a cold room. Average temperature first three weeks 32·5° F. afterwards 45° F.

Exp. XX. 200 c.c. of a mixture of two sewage samples added to 40 litres of tap-water. Feb., March, April, 1917. Average temperature 41° F.

At the end of 4 weeks and 5 weeks the *B. coli* were plated out and on each occasion both excretal *B. coli* and also the high ratio ($CO_2 : H$) *B. coli* of Clark and Lubbs were isolated.

TABLE VI. *Percentage survival.*

Examination intervals	Exp. I *B. coli*	Exp. I Streptococci	Exp. III *B. coli*	Exp. III Streptococci	Exp. XVI *B. coli*	Exp. XVI Streptococci	Exp. XX *B. coli*	Exp. XX Streptococci
Start	100	100	100	100	100	100	100	100
3 days	30	30	300	100	1000	300	33	100
7 ,,	0·3	0·3	10	1	100	300	3	33
2 weeks	0·1	0·3	0·3	0·3	30	3	1	0·3
3 ,,	0·03	0·3	0·3	0·3	10	3	0·1	0·01
4 ,,	0·03	0·3	0·1	0	1	3	0·1	0·01
5 ,,	0	0·3	0	0	0·3	10	0·3	0·01
6 ,,	0	0	—	—	0·1	3	0·01	0
7 ,,	0	0	—	—	0·03	0·03	0	0
8 ,,	—	—	—	—	0	0·1	—	—

Remarks. While the results follow the general features of the two other groups of experiments the total elimination, particularly of the streptococci, is not quite so rapid. In three out of the four experiments the number of streptococci and *B. coli* were the same at the start, making the comparative alteration specially interesting. The decline was very similar for both organisms and the parallelism close.

The frozen condition caused a prolonged survival of both *B. coli* and streptococci in Exp. XVI.

Group D. Experiments carried out with isolated strains.

Exp. V. A mixture of 10 streptococci, 6 isolated from bovine, 4 from human excreta, and 18 *B. coli* strains derived 10 from milk, 5 from sheep, 2 ox, 1 from human excreta kept in sterile tap-water. The strains were grown in broth and 4 loopfuls of each culture were added to 2 litres of tap-water in a stoppered bottle. The 112 loopfuls (about 0·2 c.c. of broth) added very little organic matter. The bottle was kept in the dark at room temperature.

The results obtained were as follows:

Examination intervals	*B. coli* (per c.c.)	Streptococci (per c.c.)
Start	1000–10,000	100–1000
1 week	10,000–100,000	100–1000
2 weeks	over 100,000	100–1000
3 ,,	100,000–1,000,000	10–100
4 ,,	over 1,000,000	0·1–1
5 ,,	,, 10,000,000	absent (from 10 c.c.)
6 ,,	1,000,000–10,000,000	,, ,,
7 ,,	10,000–100,000	,, ,,
8 ,,	100,000–1,000,000	,, ,,
9 ,,	10,000–100,000	,, ,,

Experiment not continued further. It ran from June 8th to August 18th, 1916 so that the temperature was considerable over part of this period. It was frequently between 60 and 70° F.

Exp. VI. One of the *B. coli* strains from Exp. V was isolated a month after the start of that experiment and its viability in water separately tested. 0·1 c.c. of a 24 hours' old peptone water culture was added to half a litre of sterile tap-water in flask. Kept in the laboratory in diffused light. Average temperature 60–70° F.

At start	42,000 per c.c.
End of 5 days	3,500,000 ,,
,, 12 ,,	6,000,000 ,,
,, 36 ,,	4,800,000 ,,

Although amount of nutrient material present was quite negligible the organism showed marked powers of multiplication. In its cultural characters it agreed with the ordinary excretal *B. coli* strains fermenting glucose and lactose, producing indol, clotting milk in 2 days and growing as a bluish translucent growth without liquefaction, upon sloped nutrient gelatine.

Exp. VII. Another *B. coli* strain isolated from human excreta kept in sterile tap-water; 0·1 c.c. of a 20 hours' peptone water culture being added to 1 litre of sterile tap-water and kept at room temperature in a stoppered bottle.

At start 12,700 per c.c. (July 27th).

After 6 days	102,000 per c.c.	After 21 days	100,000 per c.c.
,, 13 ,,	112,000 ,,	,, 36 ,,	162,000 ,,

The organism was still present in 1 c.c. after 147 days but was absent in 10 c.c. 40 days later.

Exp. VIII. Six streptococcus strains all recently isolated from human excreta kept in sterile water in stoppered bottle at room temperature. All short chain forms. 0·1 c.c. of a 24 hours' culture in broth added in each case.

At start	3120 per c.c.
After 8 days	2900 ,,
,, 14 ,,	1550 ,,

At the end of 21 days the number per c.c. was 10,000 but a contaminating bacillus was present so the results were unreliable and the experiment was discontinued.

Exp. XVII. A streptococcus strain isolated from cow excreta and kept in sterile tap-water in stoppered bottle at room temperature. 0·2 c.c. of a 20 hours' broth culture added to 1 litre water.

At start (Jan. 17th) 46,000 per c.c.

After 3 days	20,000 per c.c.	After 9 days	50 per c.c.
,, 7 ,,	200 ,,	,, 14 ,,	absent from 1 c.c.

Exp. XVIII. A streptococcus isolated from the fresh sewage used for Exp. XVI. 0·2 c.c. of a 24 hours' broth culture added to 1 litre of sterile tap-water. In stoppered bottle at room temperature. Jan. 20th to Feb. 6th, 1917.

At start	43,250 per c.c.	After 14 days	absent from 0·1 c.c.*
After 3 days	37,770 ,,	,, 21 ,,	,, ,, 1·0 c.c.
,, 7 ,,	18,800 ,,		

* Larger amounts not examined.

Exp. XIX. *B. coli* isolated from the fresh cow excreta used for Exp. XIII. 0·1 c.c. of a 24 hours' peptone water culture added to 1 litre sterile tap-water. In stoppered bottle at room temperature. Jan. to April, 1917.

At start	11,250 per c.c.	After 5 weeks	560,000 per c.c.
After 3 days	8250 ,,	,, 6 ,,	548,000 ,,
,, 7 ,,	4500 ,,	,, 7 ,,	456,000 ,,
,, 2 weeks	6500 ,,	,, 8 ,,	460,000 ,,
,, 3 ,,	43,000 ,,	,, 9 ,,	510,000 ,,
,, 4 ,,	95,000 ,,	,, 11 ,,	565,000 ,,

Experiment not continued further. The organism was one of the "high ratio" (CO_2 : H) type described by Clark and Lubbs, produced a definite capsule in milk, fermented lactose and saccharose well, especially the latter, and produced indol in small amount.

Exp. XXII. An experiment to test the relative viability of capsulated and non-capsulated *B. coli* in unsterilised tap-water.

One strain of each type was used and 0·03 c.c. of a 24 hours' peptone water culture was added to 1 litre of tap-water. A very hard water free from *B. coli* unsterilised and kept in separate stoppered bottles at room temperature under exactly similar conditions. The enumerations were made upon lactose neutral red bile salt agar. March, April and May, 1917.

	Non-capsulated	Capsulated
At start (per c.c.)	4400	7440
After 31 days	25	440
,, 43 ,,	3	176
,, 50 ,,	0	122
,, 57 ,,	0	81
,, 70 ,,	—	30
,, 84 ,,	—	25
,, 98 ,,	—	6

The capsulated strain was still alive in the unsterilised water at the end of 14 weeks while the non-capsulated type was dead at end of 7 weeks.

Exp. XXIV. A repetition of Exp. XXII with different strains of *B. coli* and using a different water.

One strain of each type was used and 0·03 c.c. of a 24 hours' peptone water culture was added to half a litre of water. The water used was a rather hard limestone water but free from *B. coli*. The water was used unsterilised and the samples were kept under similar conditions in stoppered bottles at room temperature. Enumerations upon lactose neutral red bile salt agar. Carried out from May 11th to June 23rd, 1917.

	Non-capsulated	Capsulated
At start (per c.c.)	8000	4920
After 14 days	960	980
,, 28 ,,	0 (absent from 1 c.c.)	540
,, 42 ,,	0 ,, ,,	29

Summary.

The experiments with individual strains in sterile water show a marked difference between the behaviour of the streptococci and *B. coli*. In all cases the streptococci diminished in numbers and in two experiments were practically eliminated at the end of 2 weeks while in the other two they did not survive very much longer. On the other hand the *B. coli* strains although in a medium which contained little or no organic matter, and which was not in any sense a nutrient medium, showed in every case multiplication, in most extensive multiplication. This was rather contrary to our anticipations as although we were prepared for prolonged survival under conditions of freedom from competing bacteria we did not anticipate such marked increase in numbers. In Exp. XIX the bacillus tested was still present over fifty-fold of the original number at the end of 11 weeks.

We have given some attention to the characters of the surviving types of *B. coli* in the tank experiments. Generally speaking they were quite normal in respect of fermentation reactions but on three occasions, Exps. XIII, XV and XX, the capsulated "high ratio" (CO_2 : H) coli of Clark and Lubbs were found, and in Exps. IV, XX and XXI the surviving organisms isolated were also capsulated but not "high ratio" organisms. It appears possible that the capacity to form capsules has something to do with the viability of these organisms and Exps. XXII and XXIV are evidence in this direction, but it is to be noted that the surviving *B. coli* in Exp. XVI after 7 weeks produced no definite capsule when grown in milk.

In Exp. XX the original sewage was plated out upon lactose neutral

red bile salt agar and 10 strains of *B. coli* isolated of which 50 per cent. showed capsule formation. Fourteen days later 11 strains were isolated from the sewage tank water and of these 90 per cent. were weak lactose fermenters and showed no capsule formation. In the same experiment 2 strains isolated after 4 weeks and another after 5 weeks showed definite capsule formation. The ultimate *B. coli* survivors were apparently all capsulated organisms, although at the start as many non-capsulated as capsulated organisms were present.

The tank experiments with excreta or sewage added to a large bulk of water yielded minor differences in the individual experiments, but in general they all show a rapid diminution and elimination of the streptococci and a continuous but not quite so rapid diminution in the number of *B. coli*. With the latter it was more common to find persistence in small numbers for a period extending to many weeks. The elimination of the streptococci was particularly uniform and rapid. At the end of 2 weeks in only one experiment were they present in more than insignificant numbers.

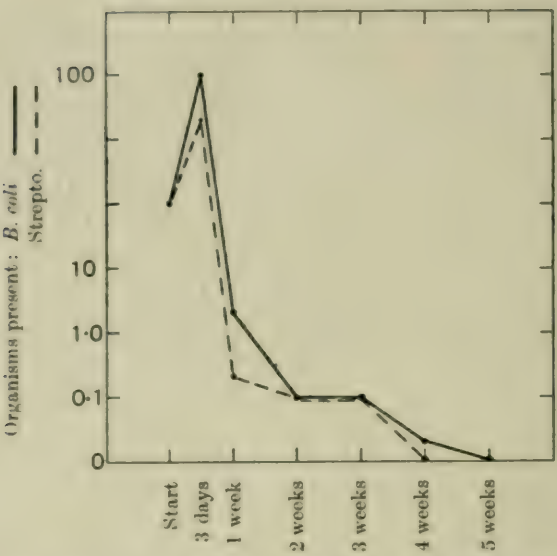
While the diminution was rather more rapid for excreta than for sewage for both streptococci and *B. coli*, no definite constant differences in relation to the kind of contamination could be made out.

The decline curves of both organisms agree very closely as can be most readily seen if the figures are plotted out as graphs. Three of the experiments, in which the initial numbers of *B. coli* and streptococci were identical, and also Exp. XI are set out in this way.

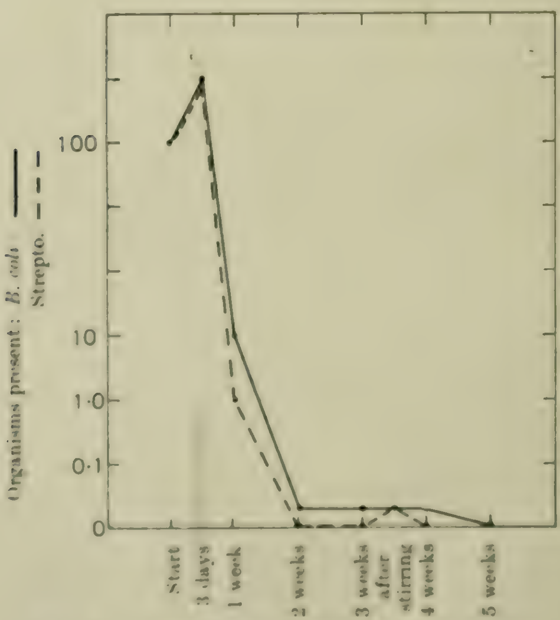
The available data is hardly extensive enough to enable deductions to be drawn other than broad and general ones, but the facts add confirmation to the view that the presence of either streptococci or *B. coli* in considerable numbers, *i.e.* in 1 or even 10 c.c. of a water, can only indicate contamination considerable in amount and of recent origin.

In particular the finding of streptococci in any numbers can be accepted as indicating considerable and recent contamination. We consider that the streptococcus determination is very valuable on its positive side as an indication of recent contamination. As a means of judging of the recency of the contamination it is even more valuable than the *B. coli* enumeration.

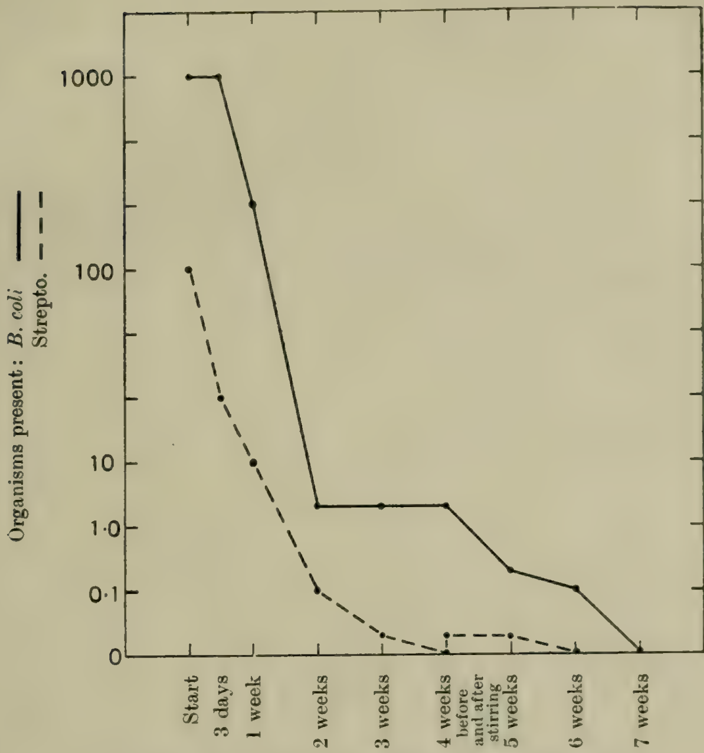
Put another way our experimental data shows that in nearly every case there is a marked diminution in the number of both *B. coli* and streptococci at the end of even 1 week, so that it follows that when these organisms are found in large numbers the contamination must have been either very recent or especially abundant.



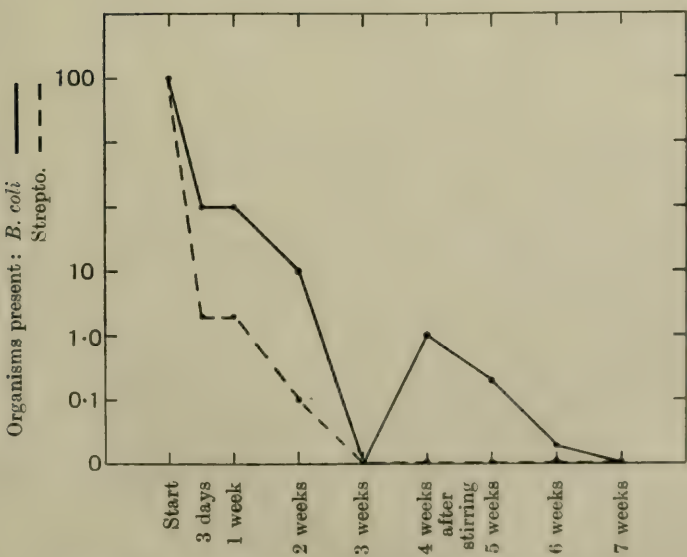
EXP. III. *Domestic Sewage.* April 17th—May 22nd, 1916.



EXP. X. *Human Excreta* (Paratyphoid Convalescent).
Nov. 6th—Dec. 11th, 1916.



EXP. XI. *Human Excreta* (Paratyphoid Convalescent). Nov. 17th, 1916—Jan. 5th, 1917.



EXP. XIII. *Cow Manure.* Dec. 12th, 1916—Jan. 23rd, 1917.

CERTAIN OBSERVATIONS ON THE ACTION OF BILE AND BILE SALTS WITH AND WITHOUT THE ADDITION OF THE SALTS OF CALCIUM ON ANIMAL RED BLOOD CORPUSCLES.

By LEONARD S. DUDGEON, F.R.C.P. (Lond.).

(*From the Department of Pathology, St Thomas's Hospital, London.*)

Human Bile.

Bile is often referred to as if its chemical composition was constant and that by treating samples of bile with extractives similar results would be obtained, whereas samples of human bile differ to such an extent that the term bile in itself signifies little. Two samples of human bile may differ to such an extent that while one sample has the qualities of true bile as regards its bile salt content the other may be entirely devoid of lytic action on red blood cells. Numerous experiments made with human bile obtained during life and from the post mortem room have shown this to be correct. The actual bile salt content of bile can be judged by boiling the bile to remove much of the protein, and also to destroy bacterial activity and then contrast the haemolytic activity of the treated sample with a 1 per cent. solution of bile salt in normal saline.

Some samples of human bile are extremely active as can be readily appreciated by a study of Table I. In addition to the action of human bile referred to in this table there is also a record of the action of 2 per cent. bile salt in saline so as to act as a control on the samples of bile which were found to be most active. It is shown here, that 2 per cent. bile salt in saline is not as active as two of the four samples of bile which are referred to.

The action of drainage of the gall bladder may be to remove the bile salt content of the bile, or more correctly what is termed bile for the first few days or may be even a week after the operation. The effect in some instances is most striking, as samples of bile which showed at

TABLE I.

Samples of actively Haemolytic Human Bile to compare with 2 per cent. Bile Salts.*

	0·5	0·45	0·4	0·35	0·3	0·25	0·2	0·15	0·1	0·08	0·06	0·05	0·03	0·01	0·005	0·001
Bile Salt 2 % in Saline. Quantity employed.	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	ICH	Tr	—	—	—
1. Gall Bladder. Diabetes. Post Mortem	CH	CH	CH	CH	CH	CH	CH	CH	ICH	D	D	Tr	—	—	—	—
2. Gall Bladder. Calculus. Operation	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	ICH	D	D	Tr
3. Gall Bladder. Calculus. Operation	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	ICH	D	Ft Tr	—	—
4. Gall Bladder. Septicaemia. Post Mortem	CH	CH	CH	CH	CH	CH	CH	CH	ICH	D	Tr	Ft Tr	—	—	—	—

In all tables saline was added to 1 c.c. and 0·05 c.c. of human red cells.

TABLE II.

The action of Human Bile on Red Cells before and after Drainage of the full Bladder.*

Amount of Bile present in each c.c. (Saline added to 1 c.c.)		1·0	0·9	0·8	0·7	0·6	0·5	0·4	0·3	0·2	0·1	0·08	0·06	0·05	0·03	0·01	0·005
1. Cholecystitis.	At operation (1) ...	—	—	—	—	—	CH	CH	CH	CH	ICH	ICH	M	D	Tr	—	—
,,	Drainage, 24 hours later (2)	—	—	—	—	—	Tr	—	—	—	—	—	—	—	—	—	—
,,	Drainage, 4 days later (3)	—	—	—	—	—	D	Tr	Ft Tr	Ft Tr	—	—	—	—	—	—	—
2. Gall Stones.	At operation	—	—	—	—	—	CH	CH	CH	CH	ICH	M	D	D	Tr	—	—
,,	Drainage, 24 hours later	—	—	—	—	—	ICH	ICH	M	D	Tr	—	—	—	—	—	—
3. Gall Stones.	At operation	—	—	—	—	—	D	Tr	Ft Tr	—	—	—	—	—	—	—	—
,,	Drainage 24 hours later	—	—	—	—	—	CH	CH	CH	ICH	M	D	Tr	—	—	—	—

* For explanation of terms, see note following Table VI (p. 246).

the actual operation a haemolytic value equal to or even greater than 1 per cent. bile salt, after operation may be reduced to a minimum, or the lytic action may be lost. Occasionally drainage may induce a flow of much more active bile than was found at the time of operation. These facts are well shown in Table II. Numerous experiments could be quoted to illustrate the wide differences which exist between the haemolytic action of various samples of human bile, but no useful purpose would be gained.

The action of Human Bile and Bile Salts on different samples of Human Red Blood Corpuscles.

The action of bile salts and human bile was tested on different samples of human red cells, e.g. normal cells, red cells from cases of obstructive jaundice and red cells from cases of congenital cholaemia, as by this means it was possible to compare the action on cells which are known to be most fragile and those which are most resistant in various strengths of sodium chloride in distilled water. No accurate distinction, however, could be obtained by the various methods employed as all human red cells appear to act in the same way with bile salt alone, or with bile salt in the presence of calcium.

The effect of boiling Human Bile.

Although boiling has no effect on the haemolytic activity of bile salts, yet, it may increase the activity of bile by removing the protein which may be present and thus permitting the bile salts to act fully. There was only one instance of a sample of bile in which the lytic action was reduced by boiling.

The stimulating effect of Calcium on the lytic action of Bile Salts.

While blood serum inhibits the haemolytic action of human and animal bile, calcium exerts a directly opposite effect (Table III). It not only excites a stimulating action in that it increases the lytic effect on red cells, but moreover it greatly increases the rate of haemolysis.

The pure crystalline preparation of calcium chloride (Kahlbaum) was used in all experiments referred to in this communication. The effect of known quantities of calcium acting on red cells in the presence of a standard solution of bile salt is fully illustrated in Table IV; also the increased rapidity of haemolysis and the accentuated action of

Table III.

To show the stimulating action of Calcium on Human Bile.

	Amount of Bile present in each c.c.	0·8	0·6	0·5	0·4	0·3	0·2	0·1	0·08	0·06	0·05	0·03	0·01	0·005	0·001
1.	Gall Bladder. Saline added up to 1 c.c.	(a) M	—	Tr	Ft Tr	Ft Tr	—	—	—	—	—	—	—	—	—
	Calculi. Saline + Ca ,, ,, ,,	(b) CH	CH	CH	ICH	D	D	Tr	—	—	—	—	—	—	—
2.	Cardiac Disease. Saline as above	(a) CH	—	CH	CH	CH	CH	ICH	D	D	M	—	Tr	—	—
	P.M. (Gall Bladder). Saline + Ca ,,	(b) CH	—	CH	CH	—	CH	CH	—	CH	CH	—	ICH	D	Tr
3.	Acute Cholecystitis. Saline as above	CH	—	ICH	—	Tr	—	—	—	—	—	—	—	—	—
	Gall Bladder. Saline + Ca as above	CH	—	CH	CH	CH	ICH	D	M	M	Tr	—	Ft Tr	—	—

Table IV.

To show the effect of the addition of varying amounts of Calcium Chloride on the rate of the Haemolytic activity of Bile Salts.

Bile Salt in Saline 1 %. Amount added	0·12	0·11	0·1	0·05	
Amount of 10 % Calcium Chloride added	0·2	0·2	0·2	0·2	Saline to 1 c.c.
Result	CH_1	CH_2	CH_3	ICH	
Amount of 10 % Calcium Chloride added	0·15	0·15	0·15	0·15	,, ,,
Result	CH_2	CH_3	CH_3	ICH	
Amount of 10 % Calcium Chloride added	0·1	0·1	0·1	0·1	,, ,,
Result	CH_2	CH_3	CH_3	ICH	
Amount of 10 % Calcium Chloride added	0·05	0·05	0·05	0·05	,, ,,
Result	CH_2	CH_4	CH_4	D	
Amount of 10 % Calcium Chloride added	0·01	0·01	0·01	0·01	,, ,,
Result	CH_{10}	CH_{13}	CH_{18}	Tr	
Amount of 10 % Calcium Chloride added	0·005	0·005	0·005	0·005	,, ,,
Result	CH_{12}	CH_{18}	CH_{18}	Tr	
Amount of 10 % Calcium Chloride added	0·001	0·001	0·001	0·001	,, ,,
Result	CH_{17}	CH_{25}	CH_{30}	Tr	
None	—	—	—	—	,, ,,
Result	CH_{20}	CH_{35}	ICH	Tr	

bile salt is fully emphasized. It is shown also in this communication how calcium increases the lytic action of bile salt on various samples of red cells and helps to distinguish between animal red cells, e.g. sheep and human. Various proportions of calcium chloride were added to the fluids which were being tested, but 0·05 c.c. of a 10 per cent. solution was generally found to be satisfactory, when the total bulk was made up to 1 c.c. It was found further that other preparations of calcium—oxide and lactate—were capable of increasing the lytic action of bile salts.

A sample of pure barium chloride (Kahlbaum) was tested in a similar manner and found to increase the haemolytic activity of bile salts or bile. Sodium chloride employed for control purposes did not accentuate the activity of bile salts except in highly "salted" solutions (10 per cent. or over). The action of calcium in the presence of bile salts is controlled by blood serum, but this is referred to elsewhere in this communication.

To compare the action of Human, Sheep, Ox, and Guinea-pig Bile on the Red Cells of these animals with and without the addition of Calcium.

In these experiments the bile was boiled for five minutes to exclude the action of living bacteria, except in the case of guinea-pig bile which was found to be sterile. In each tube a definite volume of bile was added and the whole made up to 1 c.c. with normal saline in one series of experiments, while in the other series the saline which was added contained 0·05 c.c. of a 10 per cent. solution of calcium chloride. The red cells obtained from the various animals were washed free from plasma and then 0·05 c.c. of the requisite cells was added to the mixture which was incubated at 37° C. and the results recorded at the end of every few minutes until the hour had elapsed. A standard for bile does not exist so that every sample of bile must be treated on its own merits. The bile of various animals was tested on red cells, but only human, sheep, ox, and guinea-pig bile was tested systematically. Ox bile by this means was found to be the most active bile obtainable as most samples were more efficient than a 2 per cent. solution of bile salt. Human bile varied more than animal bile in its lytic values. It was possible, however, with all samples of bile employed for this purpose to differentiate between human, sheep, pig, and ox red cells. Ox bile or bile salt in solution in saline very readily haemolyses human red cells while the action on sheep red cells is much less effective, in fact, all samples of bile and bile salts act most readily on human cells and much less effec-

tively on sheep cells. It is only necessary to compare the various samples of bile with a standard solution of 2 per cent. salt to determine the activity of the sample in question. Further this specific difference between human and sheep cells can be emphasized much more forcibly by the addition of calcium chloride to bile. It is possible that the difference between the red cells of various animals may depend on the lecithin content of the red cells as discussed in a separate paragraph in this communication.

The results of these experiments showing the action of human and ox bile on the red cells of various animals are recorded in Table V. Here also is clearly illustrated the striking difference in the action of bile salts, with and without calcium, on human and sheep red cells.

THE INHIBITORY ACTION OF BLOOD SERUM ON THE HAEMOLYTIC ACTION OF BILE AND BILE SALTS.

It has already been stated that the effect of boiling human bile is to induce a greater activity in the boiled bile or to incite no obvious change. It is well known that the addition of blood serum to bile or bile salts diminishes the haemolytic action and further such haemolysis by bile salts can be removed by the addition of sufficient blood serum. Blood serum (human or animal) can be employed for this purpose and it is a matter of no importance whether the serum is fresh or has been inactivated at 60° C., as in each instance it serves to protect the red cells from the lytic activity of the salts. We know from experience that if too much blood is employed or too weak a solution of bile salt that the chances of obtaining a culture of the *Bacillus typhosus* from the blood stream is distinctly diminished. I know of several instances in the Eastern Mediterranean in 1915 where considerable improvement occurred in blood culture technique in cases of "Enterica" by doubling the strength of the bile salts and avoiding too much blood, as by this means the "protective action" of the serum is removed and its antibacterial properties rendered void. Experiments show that the lytic action of a standard solution of bile salt on red cells can be regulated exactly by means of blood serum, while the haemolytic action of bile salt may be unopposed in the presence of the body fluids. If we take the view that blood serum serves to protect the red cells from the injurious action of the bile, then by the addition of sufficient bile salt the whole of the "protective action" of the serum will be removed and thus the growth of such bacteria as are susceptible to the action of blood serum will be free to multiply. The body fluids as shown in

TABLE V.

To compare the action of Bile Salts or Bile on the Red Cells of certain animals.

1 % Sodium Taurocholate in Saline Amount of Bile Salt employed	0·2	0·18	0·15	0·13	0·1	0·08	0·05	0·03	0·02	0·01	
1 Saline added to 1 c.c.	D	D	Tr	Tr	Ft Tr	—	—	—	—	—	Sheep cells
" "	CH	CH	ICH	M	D	D	Tr	—	—	—	Human "
0·05 c.c. of 10 % Calcium Chloride	CH	ICH	ICH	M	D	Tr	—	—	—	—	Sheep cells
and Saline added to 1 c.c. ...	*CH*	*CH*	CH	CH	CH	ICH	M	D	D	Tr	Human "

"Ox Bile." Amount of Bile employed		0·1	0·05	0·01	0·005	0·001	0·1	0·05	0·01	0·005	0·001	
2 Saline added to 1 c.c.	Human cells	*CH*	*CH*	CH	D	—	*CH*	*CH*	*CH*	CH	Tr	0·05 c.c. of Calcium Chloride
" "	Sheep cells	CH	CH	D	—	—	*CH*	*CH*	D	Tr	—	in saline added to 1 c.c.
" "	Guinea pig cells	*CH*	*CH*	ICH	Tr	—	*CH*	*CH*	*CH*	ICH	Tr	
" "	Ox cells	CH	CH	D	—	—	*CH*	*CH*	D	Tr	—	

TABLE VI.

To show the action of Blood Serum and "Body Fluids" on Bile Salt.

Bile Salt 2 % in Saline. Quantity employed. Human red cells 0·5 c.c. added to each tube.	0·45	0·4	0·375	0·35	0·3	0·25	0·2	0·1	0·05	0·03	0·01	0·005
Saline added to 1 c.c.	CH	CH	CH	CH	CH	CH	CH	CH	ICH	Tr	—	—
Human Serum. Saline added to 1 c.c.	CH	ICH	D	D	Tr	Ft Tr	—	—	—	—	—	—
Heated Human Serum " "	CH	ICH	D	D	Tr	Ft Tr	—	—	—	—	—	—
Ascitic Fluid " "	CH	CH	CH	CH	CH	CH	CH	CH	ICH	Tr	—	—
Hydrocele Fluid " "	CH	CH	CH	CH	CH	CH	CH	ICH	Tr	—	—	—
Rabbit Serum " "	CH	ICH	D	D	Tr	Ft Tr	—	—	—	—	—	—

All tubes incubated for one hour at 37° C.

Explanation of Terms. *CH* = Rapid haemolysis. CH = Complete haemolysis within 1 hour at 37° C. ICH = Almost but not complete haemolysis within 1 hour at 37° C. M = Marked haemolysis within 1 hour at 37° C. D = Distinct but less evident than "Marked." Tr = Trace. Ft Tr = Faint Trace.

Table VI are very deficient in such protective action while numerous other experiments which were completed, but are not recorded, confirm this observation on the body fluids. In most instances it is unnecessary to employ such a strong solution of bile salt in the presence of the body fluids because the protective power of these fluids is feeble.

It was found possible, with the numerous samples of human bile which were tested and found to lyse red cells actively, to control this action exactly by the addition of the necessary quantity of blood serum. For example, bile removed at operation from a case of distended gall bladder haemolysed red cells completely in one hour when a 1 per cent. solution in saline was employed, but a 40 per cent. mixture of the bile in human serum failed to completely haemolyse the same sample of red cells during the same period[1]. In Table VI, to which reference has been made, it will be seen that a sample of ascitic fluid corresponds with "saline" while a sample of hydrocele fluid is only a little more effective in preserving the red cells from the action of bile salt. These comparative results of blood serum on the one hand and ascitic fluid on the other in relation to bile salt haemolysis are very similar to those which I obtained when studying the nature of the so-called aggressive fluids and the action exerted by the anti-aggressive agents of which blood serum is the most potent.

The effect of treating Human Bile with Ether.

Bile was obtained in bulk, centrifugalised, boiled for five minutes and then centrifugalised again. The more or less clear sample of bile was then cooled and ether was added in the proportion of about 200 c.c. to 20 c.c. of bile. The ether bile was then shaken for many hours in a large glass jar at room temperature, after which the ether was separated off, while that which was more intimately mixed with the bile was removed by means of the electric fan and a warm current of air until the original bulk of bile was obtained. It was found as a result of numerous experiments that the ether treated bile is a much more powerful agent in dissolving red cells than the untreated bile. In one sample of bile it was found that a 1 in 10 dilution produced a trace of haemolysis, while the same bile after treatment with ether produced a similar effect when diluted 1 in 100. Another sample produced a trace of haemolysis with a dilution of 1 in 3, while after treatment it induced a trace of haemolysis when diluted 1 in 10. The action of ether-treated bile may depend

[1] It has been conclusively demonstrated that blood serum effectively controls bile salt haemolysis even when calcium is acting with the bile salt.

upon substances extracted from the bile which hinder the haemolytic properties of bile salts in the untreated samples, or this altered action might be explained by the assumption that ether had set free a substance which acted as a haemolytic agent in addition to the bile salts. The commercial preparation of bile salts already referred to was similarly treated with ether, but no such effect was induced as judged by the haemolytic activity of these salts in the control experiments.

Lecithin content of Red Cells.

The results recorded in this communication show that bile salt, or samples of bile, lyse the red cells of various animals with a widely different haemolytic activity. The human red cell is absolutely distinct from the sheep cell in this respect as the former is relatively fragile in the presence of these solvents. The efficiency of bile salts is greatly accentuated in the presence of calcium and it appeared probable that the explanation for this phenomenon might be an inter-action between calcium and the lecithin in the red cell. It is well known that calcium is able to precipitate lecithins, and, therefore, it was thought possible that the lecithin content of the red cells of various animals might vary. This was shown to be the case as regards human and sheep cells in the experiments arranged.

Fresh sheep and human blood was obtained in bulk into citrated saline, centrifugalised, and thoroughly washed in saline. The red cells were then collected, dried rapidly by means of an electric fan, and finally were handed to Dr Hugh Maclean, Pathological Chemist to St Thomas's Hospital, who dried them to a constant weight in a vacuum chamber, and the lecithin content was then estimated by methods which in his experience on this subject are considered to be most suitable.

The analysis completed by Dr Maclean is as follows:

Human Red Cells 2·21 mgms. per cent. of phosphorus
Sheep Red Cells 1·35 ,, ,, ,, ,,

Note:—This work has had to be abandoned for the present, but will be continued later with the hope that it may be possible to obtain definite facts of importance from the determination of the lecithin content of animal red cells and from the red cells in diseases affecting the human body.

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A COMPARISON OF MENINGOCOCCI FOUND IN THE CEREBRO-SPINAL FLUID AND NASOPHARYNX IN TWENTY-FIVE CASES.

By R. G. CANTI, M.B. (Cantab.).

Demonstrator of Pathology at St Bartholomew's Hospital.
Pathologist to the Alexandra Hospital, London, E.C.

This paper embodies the results of the routine bacteriological examination of patients suffering from cerebro-spinal fever at St Bartholomew's Hospital during the year 1916. The work was carried out in conjunction with Professor Andrewes who has already published abstracts of the first eleven cases (Andrewes, 1916).

A notable feature of the series of cases is the proportion which occurred in children under the age of two years.

Twenty-nine cases of the disease were treated in the hospital during the year. Two cases, bacteriologically proved, unfortunately died before nasopharyngeal cultures could be taken, and in two more cases meningococci could not be detected either in film or culture of the cerebro-spinal fluid, but the general characteristics of the fluid, the clinical condition of the patient, and the finding of copious growths of agglutinable meningococci in the nasopharynx, rendered the diagnosis all but certain. These four cases reduce the number under discussion to twenty-five.

History.

v. Lingelsheim (1906), working during the epidemic in Upper Silesia in 1904–5, found that in the early stages of cerebro-spinal fever positive cultures could be obtained from the nasopharynx in 93·8 per cent. of the examinations performed, provided that the material to be cultivated was dealt with immediately.

Since then other workers have met with varying success but so many have obtained a high percentage of positive results that there seems little doubt that the organism is present in the nasopharynx in the early stages of the disease in all cases.

The present outbreak which began in 1914 has again drawn attention to the organisms in the nasopharynx, one of the objects being to determine their relations with the meningococci in the cerebro-spinal fluid.

Early in 1916 Professor Andrewes (*loc. cit.*) published notes on eleven consecutive cases of cerebro-spinal fever showing that in each case the nasopharyngeal organisms were of the same agglutinable type as those in the cerebro-spinal fluid. These eleven cases are included with further details in the present series.

W. M. Scott (1916) isolated organisms of a similar agglutinable character in all but one of a series of seven cases. He further found that whereas the organisms first isolated from the nasopharynx in two of his cases were similar to the meningeal strains, those isolated at a later date did not agglutinate in the same way. He suggested that this may have been due to one of two causes, viz., either "modification may go on in the nasopharynx so that one type changes into the other" or "the later swabs were furnishing cultures of another, perhaps a normal, inhabitant of the nasopharynx which had been swamped by the infecting strain at the time of the first examination."

M. H. Gordon (1917) with the material forwarded to him from eight cases found that organisms of the same type were isolated from nasopharynx and meninges on every occasion.

M. Flack (1917) showed that similar meningococci were obtained from thirty cases in a series of thirty-two. Of the remaining two, one was found to yield organisms of different types from either site, and in the other no decision as to type was arrived at in the case of either strain.

Cultural Technique.

The medium used for primary cultures both of cerebro-spinal fluid and nasopharyngeal swabs was legumin agar prepared according to Gordon's formula with the addition of a small quantity of sterile ascites fluid and fresh human blood. In sub-cultivations the blood was omitted. For storing purposes egg medium in tubes sealed with paraffin wax as recommended by Dr A. Eastwood was used. In one case the organism was found to be alive seven months after the culture had been made, this organism having been previously repeatedly subcultivated.

Nasopharyngeal cultures were made by means of West's post nasal swab, the upturned end of the tube being greatly shortened for use on young children.

As a rule only one plate was used for nasopharyngeal cultivation, the swab being rubbed over a small area of the medium near its edge and the material so left behind distributed by means of a glass spreader, so that the intensity of the growth was graduated from one side of the plate to the other.

CULTURAL APPEARANCE.

Meningococci were obtained from the nasopharynx of every patient, and in twenty-two out of the twenty-five cases at the first attempt. In children the cultures tended to be less pure than in adults and this was attributed to the frequent presence of vomit in the nasopharynx and to the mechanical difficulty of taking the swab without contamination by saliva, the organisms of which have been shown by Gordon (1917) to inhibit the growth of meningococci. The approximate percentages of meningococcus-like organisms present in the nasopharyngeal cultures is shown in the table.

No difficulty was experienced in obtaining a growth from the cerebro-spinal fluid.

Except in one case the colonies both in primary culture and sub-culture exhibited the same appearances as are usually described and these need no comment. In one case, however, No. 19, the colonies from the cerebro-spinal fluid on both of the only two occasions on which it was cultivated, appeared after twenty-four hours' incubation, as minute translucent points easily overlooked at a casual glance. Subsequently a few of the colonies increased in size till they appeared like average normal colonies, but the bulk, though slightly increasing in size, remained minute. Sub-cultivations from small colonies through a large number of generations behaved in exactly the same way as the original cultures, while sub-cultivations from the large colonies yielded large colonies only.

A fuller description of this organism will be published at an early date.

AGGLUTINATION.

Pure twenty-four hour cultures of the organisms grown on legumin agar with the addition of ascites fluid but without blood were emulsified in a small quantity of physiological saline solution. These emulsions were heated to 65° C. in a water bath for half-an-hour and then diluted to a concentration of about 2000 millions per c.c., one-tenth of the volume of a 5 per cent. solution of phenol in distilled water being afterwards added.

The macroscopic method of agglutination was adopted. The dilutions of 1 in 50, 1 in 100, 1 in 200 and 1 in 400, were made in quarter inch Durham's tubes, the concentration of organisms in each tube being 1000 million per c.c. The results were recorded after heating for twenty-four hours in an oven at 55° C.

With the exception of Type II S.B.H. serum, which was prepared at St Bartholomew's Hospital partly from the cerebro-spinal and partly from the nasopharyngeal coccus of the same patient (No. 2), all the sera employed, which were kindly supplied by Lt-Col. Gordon and Dr F. Griffith, were monovalent preparations from spinal strains. Gordon's sera were supplied having a titre of 1 in 400 with the homologous coccus, whilst Griffith's sera and Type II S.B.H. serum were of somewhat higher potency.

Time after onset of illness at which the meningococcus was first isolated from the pharynx.

Patients were swabbed as soon as possible after diagnosis. Meningococci were found in the first week of the disease in sixteen cases, in the second week in four cases, in the third week in three cases, and in the fourth week in two cases. These figures include the three cases in which meningococci were not obtained at the first attempt; they were eventually obtained from two of them in the third week and from one in the fourth week.

Carbohydrate Reactions.

The carbohydrate reactions were carried out in 1 per cent. glucose and in 1 per cent. saccharose ascites litmus broth on both the meningeal and nasopharyngeal cocci in sixteen cases. Fermentation was always obtained in glucose and never in saccharose. The time taken for the red colour of the glucose tubes to reach its greatest intensity was from three to six days. It was observed that in ten cases the nasopharyngeal organism took the same time to ferment glucose as the meningeal, and in all but one of the remaining cases the nasopharyngeal organism formed acid more rapidly.

In one case (No. 7) the glucose tubes both of the meningeal and nasopharyngeal coccus were first turned red and then bleached yellow by the fifth day, the red colour returning in each case on the seventh or eighth day. This investigation was performed on two occasions and controlled by other tubes of the same batch.

Types of meningococci obtained from the cerebro-spinal fluid.

Reference to the table shows that all organisms obtained from the cerebro-spinal fluid were agglutinated with at least one of the sera employed, and they are thereby divided into types as represented by Type I, Gordon, and Group I, Griffith on the one hand, and Type II, Gordon, Group II, Griffith, and Type II, S.B.H. on the other. Most of the organisms which were agglutinated by sera of the first type, were also agglutinated by Type III serum, Gordon, but usually to a less degree. In the absence of further proof they have been considered as belonging to Type I.

The proportion of the types found is as follows:

Type I	7 cases	28 per cent.
Type II	18 cases	72 per cent.

Children.

The number of children examined under two years of age was ten.

No appreciable differences in the proportion of types among them and among older patients were shown. The total number of cases examined however is too small to permit of an accurate conclusion being drawn on this point. Type I was yielded by three children (30 per cent.) and seven older patients (27 per cent.) and Type II by four children (70 per cent.) and eleven older patients (73 per cent.).

Comparison of agglutinability of the meningococcus isolated from the cerebro-spinal fluid and nasopharynx of the case.

Referring again to the table a striking similarity of agglutinability of the cerebro-spinal and nasopharyngeal cocci is seen. Firstly, the two are without exception shown to be of the same agglutinable type. Secondly, in a large number of instances a serum is found to agglutinate the nasopharyngeal coccus to the same titre as the cerebro-spinal coccus although that titre may not be the same as with the homologous coccus. This point is particularly well brought out by certain of the poorly agglutinable organisms which are entirely untouched by one or more of the sera of a particular type and yet are agglutinated by others. Examples of this are furnished by Nos. 8, 11 and 22.

In the exceptional instances there is no indication as to whether the nasopharyngeal cocci are on the whole more or less agglutinable than the cerebro-spinal.

					SERA													
					Littledale or Mitchell Type I Gordon		M 17 Group I Griffith		Chase Type III Gordon		Glidden Type II Gordon		Waterman Type II Gordon		M 24 Group II Griffith		Thomson Type II s B.H	
Case No.	Age, Sex	Source of organism	Day of isolation of organism	Roughly % of meningococcus-like organisms in cult. of Ph.	1 : 200	Highest titre	1 : 200	Highest titre	1 : 200	Highest titre	1 : 200	Highest titre	1 : 200	Highest titre	1 : 200	Highest titre	1 : 200	Highest titre
1	44 ♀	Sp.	4	.	+	>400	.	.	−	50	−	−	.	.	.	.	.	.
		Ph.	8	99	+	>400	.	.	+	>400	−	−	.	.	.	.	.	.
2	21 ♀	Sp.	2	.	−	−	.	.	−	−	−	−	.	.	.	.	+	>400
		Ph.	7	5	−	−	.	.	−	−	−	−	.	.	+	>200	.	.
3	11 ♂	Sp.	4	.	+	>400	.	.	+	>400	−	−	.	.	.	.	.	.
		Ph.	9	10	+	>400	.	.	−	−	−	−	.	.	.	.	.	.
4†	33 ♀	Sp.	2	.	−	−	.	.	−	−	−	−	.	.	.	.	.	.
		Ph.	3	90	−	−	.	.	−	−	−	−	.	.	.	.	.	.
5	3 ♀	Sp.	2	.	−	−	.	.	−	−	.	.	.	.	.	.	+	50
		Ph.	7	5	−	−	.	.	−	−	.	.	.	.	+	>400	+	50
6	24 ♂	Sp.	1	.	−	−	.	.	−	.	.	.	.	.	.	.	+	200
		Ph.	2	1	.	.	.	.	.	−	.	.	.	.	.	.	+	200
7	$\frac{5}{12}$ ♂	Sp.	2	.	−	−	.	.	−	−	.	.	.	.	.	.	+	100
		Ph.	2	5	−	−	.	.	−	−	.	.	.	.	.	.	+	100
8	7 ♂	Sp.	2	.	−	−	.	.	.	.	−	100	−	−	+	>400	−	−
		Ph.	2	60	−	−	.	.	.	.	−	50	−	−	+	>400	−	−
9	17 ♀	Sp.	2	.	−	−	.	.	−	−	+	200	+	200	+	>200	+	200
		Ph.	3	50	−	−	.	.	−	−	+	200	+	200	.	.	+	200
10	$\frac{7}{12}$ ♀	Sp.	10	.	−	−	.	.	−	−	+	>400	+	>400	.	.	+	>400
		Ph.	20	5	−	−	.	.	−	−	+	>400	.	.	.	.	.	.
11	$\frac{5}{12}$ ♂	Sp.	17	.	−	−	.	.	−	−	−	−	−	−	+	>400	−	−
		Ph.	17	30	−	−	.	.	−	−	−	−	−	−	+	>400	−	−
12	1½ ♂	Sp.	4	.	−	−	.	.	−	−	+	>400	.	.	.	.	.	.
		Ph.	4	75	−	−	.	.	−	−	+	200	.	.	.	.	.	.
13	1½ ♀	Sp.	3	.	+	>400	.	.	+	200	−	−	.	.	.	.	.	.
		Ph.	3	50	+	>400	.	.	−	100	−	−	.	.	.	.	.	.

14	1¼♀	Sp.	22	.	+	>400	.	.	+	200	–	–	.	.	.	.	.	.
		Ph.	22	20	+	>400	.	.	+	>400	–	–	.	.	.	.	.	.
15	10♀	Sp.	6	.	–	–	.	.	–	–	+	200	–	50	+	>400	+	200
		Ph.	7	60	–	–	.	.	–	–	–	–	–	50	+	>400	–	–
16	18♂	Sp.	6	.	–	–	.	.	–	–	–	100	.	.	+	>400	.	.
		Ph.	7	<1	–	100	.	.	–	–	+	200	.	.	.	.	.	.
17	2♀	Sp.	4	.	–	–	.	.	–	–	+	200	.	.	.	.	.	.
		Ph.	4	25	–	–	.	.	–	–	+	200	.	.	.	.	.	.
18	2¾♀	Sp.	4	.	–	–	.	.	–	–	–	100	.	.	–	100	.	.
		Ph.	6	50	–	–	.	.	–	100	–	50	.	.	–	100	.	.
19	6/12♀	Sp.	23	.	–	–	.	.	–	–	.	.	.	.	+	200	.	.
		Ph.	23	50	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
20	2/12♀	Sp.	7	.	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
		Ph.	7	10	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
21	3♂	Sp.	19	.	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
		Ph.	19	10	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
22	36♂	Sp.	4	.	–	–	+	>400	–	100	.	.	.	.	–	50	.	.
		Ph.	18	2	–	–	+	>400	–	100	.	.	.	.	–	50	.	.
23	4♀	Sp.	4	.	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
		Ph.	4	20	–	–	.	.	–	–	.	.	.	.	+	>400	.	.
24	1♂	Sp.	10	.	+	>400	+	>400	–	100	.	.	.	.	–	50	.	.
		Ph.	21	20	+	>400	+	>400	–	–	.	.	.	.	–	–	.	.
25	1¾♀	Sp.	11	.	.	.	–	–	.	.	.	.	–	–	+	>400	.	.
		Ph.	12	10	.	.	–	–	.	.	.	.	–	50	+	>400	.	.

The cases are arranged in chronological order.

\+ indicates the presence of agglutination as evidenced by marked flocculi when viewed with the hand lens.

\> indicates that agglutination was present at the titre indicated but that this does not necessarily represent the highest titre.

Sp. indicates that the organism was isolated from the cerebro-spinal fluid.

Ph. indicates that the organism was isolated from the nasopharynx.

† These organisms have been shown by Lt-Col. Gordon to belong to Type II.

Summary.

In twenty-five consecutive cases in which nasopharyngeal cultures have been made from patients suffering from bacteriologically proved cerebro-spinal meningitis, meningococci have been obtained.

A striking similarity between the organisms from the two sites has been shown to exist for not only are they of the same agglutinable type but in the majority of instances individual peculiarities of agglutination have corresponded. The closeness of relation has been further demonstrated by the carbohydrate reaction in one case in which the unusual property of bleaching glucose litmus broth to a yellow colour was possessed by both organisms. Nevertheless, in a certain number of instances, though organisms of a different agglutinable type have not been isolated, as by other workers, there have been found certain minor variations of agglutination. That most if not all of these variations are due to alteration of the agglutinable capacity of one of the organisms seems probable, as in the six cases where most marked difference existed (Nos. 1, 3 and 24 of Type I and Nos. 15, 16 and 18 of Type II) the chances of the other alternative, namely that a second infection had happened in each case to be of the same agglutinable type, are remote.

In the twenty-five cases the proportion of the types present has been shown to agree with that found by other workers during the same period. Seven of the infections (28 per cent.) have been due to Type I and eighteen (72 per cent.) to Type II.

Ten of the cases have occurred in children under the age of two years, and the proportion of the two types among them has been approximately the same as among older patients.

The writer is indebted to the members of the staff of St Bartholomew's Hospital for permission to publish their cases, and gratefully acknowledges the encouragement and assistance of Professor Andrewes at whose instigation these investigations were conducted.

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TYPES OF DYSENTERY BACILLI ISOLATED AT No. 3 AUSTRALIAN GENERAL HOSPITAL, CAIRO, MARCH—AUGUST, 1916, WITH OBSERVATIONS ON THE VARIABILITY OF THE MANNITE FERMENTING GROUP.

By C. J. MARTIN, F.R.S., Lt-Col. A.M.C.,

AND

Sister F. E. WILLIAMS, Australian A.N.S.

The results of attempts to isolate Dysentery bacilli from 217 cases in which the stools contained muco-pus with or without blood are tabulated below. In many cases the amount of mucus in the stool was very small. All these cases occurred amongst Australian troops of the E.E.F.

Table I.

Amoeba histolytica or cysts present		63
B. dysenteriae Shiga isolated		47
Mannite fermenting dysentery bacilli	Agglutinated at time of isolation by Y serum*	64
	Not agglutinated at time of isolation by Y serum	12
No causative organism recovered		36
	Total	222

* Serum made from Hiss and Russell's Y bacillus at the Lister Institute, original titre 1/8000.

In five instances dysentery bacilli were isolated from patients with amoebic infections.

The method employed was to wash the mucus, break it up in sterile broth and plate off some drops on the surface of a MacConkey plate. Next day likely colonies were picked off, sown into warm broth and incubated for a few hours. They were examined for motility and if motile discarded. The non-motile broth cultures were sown into glucose, lactose and mannite peptone water and on to an agar slope. If glucose or both glucose and mannite were fermented with the formation of acid only and lactose unchanged an emulsion was made from the agar

slope and tested as regards agglutinability against a Shiga or Y serum respectively. Macroscopical methods were employed.

In forty-nine instances the further biochemical characteristics of the strains were investigated immediately.

The Shiga types of dysentery bacilli isolated were all true to type and agglutinated as a rule well at a dilution of 1/800 with a serum made

TABLE II.

Variation in the Fermentations, etc., of organisms agglutinated by Flexner-Y Serum.

	Maltose	Saccharose	Dextrin	Raffinose	Arabinose	Isodulcite	Sorbite	Glycerin	Indol
1	A	O	A	A	O	O	O	O	+ +
2	O	O	A^4	A	A^6	O	O	O	+ +
3	A	O	O	A	O	O	O	O	O
4	O	O	O	A	A^4	O	O	O	O
5	O	O	O	O	A^4	O	A	O	+ + +
6	O	O	O	A	A^6	O	O	O	O
7	A	O	O	O	O	O	A	O	+ + +
8	A	O	O	O	A^4	O	A	O	+ + +
9	O	O	O	A	O	O	O	O	+ +
10	O	A^7	O	A	O	O	O	O	O
11	O	O	O	O	O	O	O	O	O
12	O	O	O	A	O	O	A	O	+
13	O	O	O	A	O	O	O	O	O
14	A	A^6	A	A	O	O	O	O	+
15	O	O	O	A	O	O	O	O	+
16	O	O	O	A	O	O	O	O	+
17	O	O	O	A	O	O	O	O	+
18	O	O	—	O	—	O	A	O	+ + +
19	A	O	—	O	—	O	A	O	+ + +
20	A	O	—	O	—	O	A	O	O
21	A	O	—	O	—	A^3	O	O	O
22	A	O	—	A	—	O	O	O	+
23	O	O	—	O	—	O	O	O	O
24	A	O	—	O	—	O	O	O	+
25	O	O	—	A^5	—	O	A	O	O
26	A	O	—	O	—	O	O	O	+ + +
27	O	O	—	O	—	O	A	A^9	+ +
28	A	O	—	O	—	O	O	O	O
29	O	O	—	O	—	O	O	O	O
30	A	O	—	A	—	O	O	O	+
31	O	O	—	O	—	—	A	O	+
32	O	O	—	O	—	—	A	O	+
33	O	O	—	A	—	—	O	O	+
34	O	O	—	A	—	—	O	O	O
35	O	A^7	—	A	—	—	O	O	O
36	O	O	—	O	—	—	O	O	O
37	O	O	—	A	—	—	O	O	+

at the Lister Institute. Higher dilutions were not tried as the serum, although specific, had not a high titre for the homologous organism at the time we were using it.

The organisms of the mannite fermenting group were, on the contrary, very variable, both as regards their biochemical activities and agglutinability. Forty-nine of them were tested upon a variety of carbohydrates. They all failed to form acid in lactose, dulcite, inulin and adonite. They all produced acid in glucose, mannite and galactose and turned milk first acid then alkaline. Their action upon maltose, saccharose, dextrin, raffinose, arabinose, isodulcite (rhamnose), sorbite and glycerin and their power to form indol varied with different strains and the same strain varied at different times. The details of these variable reactions are given in Tables II and III. Table II includes all those which at the time of isolation were agglutinated by our Y serum, Table III those which were not.

TABLE III.

Variable Fermentations, etc., of organisms not agglutinated by Y Serum at the time of isolation.

	Maltose	Saccharose	Dextrin	Raffinose	Arabinose	Isodulcite	Sorbite	Glycerin	Indol	Agglut. by Patient's serum	Agglut. by polyvalent curative serum	Agglut. by Y serum 6 months after isolation
38	A	O	—	A	—	O	O	A^3	+	1/400	1/250	O
39	A	O	—	A	—	A	O	A^3	+	O	—	1/1600
40	A	O	—	O	—	—	O	O	O	O	—	—
41	A	O	—	O	—	A	O	O	+	—	—	—
42	A	O	—	O	—	—	O	O	+	—	—	—
43	O	O	—	O	—	O	O	A^5	O	O	O	O
44	O	O	—	O	—	—	O	O	O	—	—	—
45	A	O	—	O	—	A	O	O	O	1/200	—	1/800
46	A	O	—	O	—	A	O	A^9	O	1/800	—	1/400
47	O	O	—	O	—	O	O	O	+	O	—	1/1600
48	A	O	O	—	A	A	A	A^4	O	1/400	1/250	O
49	O	O	—	O	—	O	A	O	+	—	O	O

A = Acid only in peptone water containing the various carbohydrates in 24 hrs.
A^3 = ,, ,, ,, ,, ,, ,, ,, ,, ,, 3 days.
O = Growth but no change in reaction.
— = Not tried.
+ = Indol formation determined by paradimethylaminobenzaldehyde and for + + and + + + strong reaction of indol with above reagent.

Thirty of the cultures in Table II were kindly re-examined for us by Miss Rhodes of the Lister Institute, about six months after isolation. Meantime they had been subcultured at intervals on agar slopes. Out of the three originally fermenting saccharose two had lost this property.

Five had lost the power to split maltose and other five had acquired it; four had lost the power to split sorbite, and the other two had acquired it. In six cases the action upon raffinose was reversed, three ceasing to ferment this sugar and three gaining this faculty.

The proportion of indol was the most variable; the property being lost in nine cases and acquired in four.

The instability in biochemical activities manifested by these Egyptian strains, other than in the action upon lactose, dulcite, glucose and mannite, is in accordance with observations upon organisms of this group isolated in different parts of the world, and a survey of the voluminous literature on this subject convinces us that any attempt to separate the mannite fermenting dysentery bacilli into groups on the ground of their action upon carbohydrates is unsound.

Fermentation of Saccharose.

Strong (1900) isolated a bacillus from cases of dysentery in the Philippines which differed from Flexner's organism derived from the same source in not fermenting maltose but attacking saccharose. On these grounds it has been regarded as a new species of dysentery bacillus but we understand from Prof. Strong that he is not of this opinion.

The inference that saccharine fermentation differentiates Strong's from Flexner's bacillus was disturbed when Hiss (1904) found that at this time two out of three strains of the latter fermented saccharose in peptone water more readily than the former, twelve days elapsing in the case of Strong's bacillus before the development of acidity.

There are two strains of Strong's bacillus at the Lister Institute which have been propagated for several years. When one of us had occasion to examine these two strains a few years ago they would neither of them attack saccharose. We notice also that Fraser (1916) says that a culture of Strong's bacillus obtained by him from the Director of the Bureau of Science, Manila, failed to ferment saccharose. The capacity to split cane sugar is obviously a variable characteristic, with a tendency to disappear upon laboratory media. Three of our cultures fermented saccharose at the time they were isolated and two retained this power after six months. The property may more quickly disappear. Hehewerth (1916) found that three out of twenty six strains isolated during a small epidemic fermented saccharose but all lost this faculty four months later. Fraser (1916), too, gives instances to the same effect, in which the faculty had disappeared in fourteen days.

So far we have been principally concerned with the disappearance of the power to split cane sugar. By artificial selection strains possessing this characteristic may also be derived. Twort (1907) propagated a Flexner's bacillus, which at the time did not ferment saccharose, upon relays of peptone water containing this sugar, subculturing about every fourteen days. After some months the strain was found to possess the characteristic of readily fermenting saccharose.

The same faculty may arise spontaneously. In 1915 it was noticed that the strains of Flexner at the Lister Institute were inconstant as regards the fermentation of saccharose and maltose in peptone water and at the same time displayed a tendency to form secondary colonies[1].

Variations in carbohydrate fermentation were studied in detail by Massini (1907) and Burk (1908) and Kowalenko (1910) with Neisser's (1906) *Bacterium coli mutabile*. Lactose fermentation is, however, the stable character of this organism, acid-forming colonies breeding true and non-acid-forming colonies breeding both kinds. It seemed more likely that the nature of the character of saccharose fermentation would, in the case of dysentery, more closely resemble the isodulcite fermentation of typhoid and dysentery bacilli investigated by Müller (1908, 1911) and by Penfold (1911), and the lactose fermentation of typhoid bacilli by Penfold (1911). In these latter cases the faculty to ferment the sugar is the variation from type. The white colonies on Endo or MacConkey's media breed true and the red colonies mixtures, and on subculture on to media devoid of the particular sugar the faculty becomes undiscoverable after a few subcultures.

In the case of dysentery bacilli, however, even when propagated on broth media, the fermentation of saccharose may again, for reasons unknown, become a characteristic of the strain of sufficient prominence to be observable.

We investigated one of our strains which at the time of isolation produced acid in peptone water after a few days. Our object was to determine whether the faculty could be enhanced by selection and whether it possessed any stability. We were unfortunate in our choice for of the three strains originally fermenting saccharose this particular one soon lost this power, whereas the others have retained it during six months.

To increase the number of microbes in the culture capable of fermenting the sugar we used the method of Neisser (1906).

[1] Personal communication by Dr Harriette Chick.

17. VI. 16.	Sown in saccharose peptone water.
24. VI. 16.	Acid reaction developed.
27. VI. 16.	The 10th day, plated on neutral red saccharose agar.
28. VI. 16.	50 pink colonies; 800 colourless colonies which remained so five days. *Subcultures from colourless colonies bred only colourless colonies, which remained colourless.*
	Two pink colonies sown in saccharose peptone water.
3. VII. 16.	One of the peptone waters developed acid in five days, other not. From former a plate of saccharose neutral red agar made.
4. VII. 16.	300 colonies.
6. VII. 16.	All pink.
7. VII. 16.	18 *colonies have developed daughter colonies of bright red colour.* Red bud sown in peptone water.
8. VII. 16.	Peptone water strongly acid. Loopful planted on saccharose agar.
9. VII. 16.	350 colonies developed.
10. VII. 16.	All colonies pink; 50 developing buds.
11. VII. 16.	Buds well grown and bright red. Bud sown in saccharose peptone water and on to another saccharose plate.
12. VII. 16.	Peptone water acid in 16 hours. About 800 colonies on plate all pink.
14. VII. 16.	45 colonies developed red buds.
15. VII. 16.	Material from a bud plated on to saccharose agar.
18. VII. 16.	Numerous colonies developed red buds but the colonies were too thick for enumeration.
	One bud sown in saccharose peptone water.
19. VII. 16.	Peptone water acid in 15 hours.
	At this stage the process of artificial selection was suspended as we were apparently not increasing the character. We had long since ceased to get any colourless colonies. All colonies turned pink, but the proportion developing secondary colonies remained about the same.
20. VII. 16.	Material from a bud was sown into broth and subcultured every few days for one month.
20. VIII. 16.	Broth culture sown in saccharose peptone water and plated on saccharose neutral red agar.
21. VIII. 16 to 31. VIII. 16	No acid formed in 10 days in the peptone water. Only permanently colourless colonies developed upon the saccharose plate.

In this strain the power to ferment cane sugar was a feeble characteristic, tending to disappear and only developed and maintained by natural or artificial selection.

During the course of this experiment an interesting and unusual change in the agglutinability of the organism occurred. On the 4. VII. 16 it was agglutinated completely by a dilution of 1 in 800 of our Y serum. On the 9. VII. 16, that is, after we had selected from daughter

colonies, it was retested and was agglutinated equally well, but on the 21. VII. 16 it was not agglutinated by a dilution of 1 in 50. This was repeated and the serum we were using tested and there was no apparent source of error. The non-agglutinable culture was kept and propagated upon nutrient agar, being subcultured at fortnightly intervals. A few months later it was retested and found to agglutinate as well as ever.

As far as we are aware, variations, whether natural or induced, in the fermentative characters of bacilli are not usually accompanied by serious alterations in their agglutinability. Kowalenko (1910, p. 289), Penfold (1911, p. 51).

Jacobsen (1910) has, however, recorded an instance of daughter colonies possessing different agglutinability to the mother colonies in the case of his *Bacterium typhii mutabile* which was isolated from cases of typhoid fever in a lunatic asylum. The mother colonies of this organism were inagglutinable by typhoid serum, whereas the daughter colonies were agglutinated in a dilution of 1 in 10,000. Further, by propagating from single bacilli of the mother colonies in broth, agglutinability gradually developed until in four months the full sensitiveness was reached.

Fermentation of Maltose.

Maltose and saccharose fermentation were properties utilized by Lentz (1913) to differentiate mannite fermenting dysentery bacilli into groups. To what extent this is likely to be useful in the case of saccharose has been just discussed. The fermentation of maltose is a considerably less stable property of these dysentery bacilli than that of saccharose. Hiss (1904) showed that his Y bacillus fermented maltose after successive cultures on media containing this sugar. In our observation one-third of the strains examined had in six months changed in this respect. Hehewerth (1916) examined his cultures at intervals up to four months after isolation and found that in twelve out of twenty-six the action was reversed, some acquiring the faculty to ferment maltose, others losing it. Individual colonies from the same patient behaved differently.

Other Carbohydrates. With the remaining carbohydrates, sorbite, raffinose, isodulcite and dextrin, the action is uncertain and varies from time to time. Indol production is equally capricious.

The action of the organisms isolated in Egypt upon isodulcite demands a short comment. It will be noticed that but one in Table II

fermented this sugar and that late, whereas five out of the twelve which were not, at the time isolated, agglutinated by Y serum, did so.

Isodulcite is only exceptionally fermented by any of the Flexner or Y strains, but, as shown by Müller (1908), by growing the former upon agar containing this pentose, daughter colonies may be formed which readily attack it. On the other hand Morgan (1911) found that a strain of Strong's bacillus *B. pseudo-dysenteriae* D Kruse and some of Ruffer and Willmore's (1909) El Tor strains produced acid in isodulcite with varying rapidity.

Morgan also examined the cultural characteristics of a number of strains of dysentery-like organisms fermenting mannite which Ledingham had isolated in the course of an extensive investigation upon typhoid carriers in Great Britain. In no case was there any reason to suppose that the individuals from whom they were derived were suffering or had suffered from dysentery. The majority of these strains fermented isodulcite and about half of them were agglutinated by Y serum in high dilution.

We come to the conclusion therefore that for purposes of isolation and identification of the dysentery bacilli, the only carbohydrates of service are glucose, mannite, lactose and dulcite.

The Agglutination of the Mannite fermenting dysentery bacilli.

Hiss (1904) divided these bacilli into three groups, Y, Strong and Flexner, by serological reaction. He showed that a serum made with his Y bacillus agglutinated Flexner's bacillus nearly as well as the homologous serum. On the other hand, a serum made with Flexner's bacillus did not agglutinate the Y bacillus in high dilution. The agglutinins for the Flexner bacillus in a serum made from Y bacillus were completely absorbed by an emulsion of Flexner's bacillus, leaving those for Y intact. Reciprocally Y bacilli only absorb the agglutinins for Y out of a Flexner serum, leaving the titre for the Flexner bacillus undiminished.

Kruse (1907) found at least six groupings necessary to accommodate the strains he examined, the sixth being a cave of Adullam for those which would not comfortably fit into one or other of the first five groups. The majority of organisms isolated from epidemics in Germany, up to this time, fell into either his Group A or D. Kruse suggested that his Group D is the same as that represented in America by the Y bacillus of Hiss and Russell but had not a culture of the latter for comparison.

Morgan (1911), however, found that although Y serum agglutinated *B. pseudo-dysenteriae* A Kruse and one specimen of *B. pseudo-dysenteriae* D Kruse to the full titre of 1/20,000 of the serum and another specimen to 1/500 only, in no case did these three bacilli remove the agglutinin for Y. *B. pseudo-dysenteriae* D Kruse must therefore be regarded as serologically distinct from Y.

The agglutinations of Egyptian "El Tor" strains of dysentery were examined by Ruffer and Willmore (1909). From experiments with an El Tor serum, a Flexner serum and a *B. pseudo-dysenteriae* D serum, Ruffer and Willmore placed the mannite fermenting dysentery bacilli into two groups. The first contains El Tor No. 1 and *B. pseudo-dysenteriae* D Kruse, and in the second they place *B. dysenteriae* Flexner and *B. pseudo-dysenteriae* A Kruse. The members of each group could, however, be differentiated by absorption experiments.

Later, Morgan (1911) studied El Tor bacilli, *two years after their isolation*, along with other well-known strains, and found that the former as well as the latter were all agglutinated in a dilution of 1/2000 of a serum he prepared from a specimen of Hiss and Russell's Y bacillus and, with one exception, by a Flexner serum but in a much lower dilution.

Winter (1912) also examined thirty-one strains of this type of dysentery bacillus; thirty were derived from German sources and one was a Flexner bacillus obtained from America. He found Castellani's absorption method unsatisfactory and confined his observations to cross agglutination experiments. On this basis eleven of his strains appear to correspond to Kruse's *B. pseudo-dysenteriae* D and seven to Kruse's A Group. Three are so poorly agglutinated by any of his sera that it is uncertain how they should be classed, but they appear to belong to Group A. Two are intermediate between Groups A and D, and the remaining twelve cannot be grouped by the sera employed.

Hutt (1913) emphasizes the futility of grouping dysentery bacilli according to their action upon carbohydrates. This had previously been pointed out by Kruse. He used the absorption method and thereby confirmed Kruse's groupings, indeed increasing their number.

The position may be summed up by saying that the mannite fermenting dysentery bacilli comprise a number of strains serologically distinguishable by the absorption method, but overlapping considerably as regards agglutination. For the diagnosis of an organism suspected to belong to this dysentery group no one serum is adequate; sera made with a member of each of Kruse's groups A, D and E, would embrace

most of the German strains. Y serum seems to be least specific and covers the greatest range.

From Morgan's work Y serum seemed to be particularly indicated for diagnosis work in Egypt, and we used it exclusively. Nevertheless, nearly one-sixth of the bacilli we recovered in 1916 were not agglutinated by Y serum directly after isolation. The titre of our serum against the homologous organism was 1/4000 at the time of using.

Eight of these strains were kindly re-examined by Miss Rhodes six months later. Four of them were then agglutinated by the same Y serum in dilutions, varying from 1/400 to 1/1600, so that in the meantime they had acquired some sensitivity by cultivation. Two of the four strains which still failed to be agglutinated were clumped by the polyvalent curative serum of the Lister Institute[1] in a dilution of 1/250.

In eight of the cases we were able to test the organism isolated against the patient's own serum and in four of them it was agglutinated in dilutions above 1/100 (see Table III), indicating that he had been infected by the microbe recovered from his stool. Two of the strains which did not acquire agglutinability on culture are included amongst those which were agglutinated by the patient's serum.

It would be wrong to assume that a bacillus with the morphological, cultural and biochemical characters of the Flexner group of dysentery bacilli is discredited as an etiological factor because it is not agglutinated by Y or any other univalent serum. This group of organisms is clearly in an unstable condition and records frequently occur of strains which are not agglutinated by sera prepared from the common type-strains. A recent instance, which is interesting on account of the unusual toxicity of the strain for rabbits, is given by d'Herelle (1916). This organism, which possessed the usual characteristics of the Flexner group, was isolated in five cases during a small epidemic amongst a troop of Dragoons and was not agglutinated by the polyvalent Flexner Y serum of the Pasteur Institute.

Summary and Conclusions.

1. Of 123 dysentery bacilli isolated, forty-seven were *B. dysenteriae* Shiga, seventy-six were mannite fermenters.
2. The biochemical activities of forty-nine strains of the latter were investigated immediately after isolation and again six months later.

[1] In the process of immunization the horses furnishing this serum had been injected with a number of Egyptian strains received from Sir Armand Ruffer some years ago.

They showed variability as regards the fermentation of maltose, saccharose, dextrin, raffinose, arabinose, isodulcite, sorbite and glycerin and in the formation of indol. The same strain varied at different times, some gaining, others losing one or other of the above properties.

Similar observations by other observers and the authors are discussed and the conclusion is arrived at that the separation of the mannite fermenting dysentery bacilli into groups on the ground of their action upon the above carbohydrates is unsound and that the only carbohydrates of service for their identification are glucose, mannite, lactose and dulcite.

3. An experiment conducted during two and a half months upon a particular strain of dysentery bacillus shows that the fermentation of saccharose was in this strain a "recessive character" and only maintained by artificial selection.

4. One-sixth of the mannite fermenting dysentery organisms isolated were not agglutinated by a univalent Y (rabbit's) serum at the time of isolation but half of these acquired this property by cultivation. Others were well agglutinated by the patient's serum. An experiment is described in which agglutinability to Y serum was lost under prolonged cultivation on saccharose peptone media. In this experiment successive cultures were made every few days and the material for subcultivation was taken from daughter colonies (buds), which arose in the saccharose plates. Agglutinability was rapidly regained by propagation in broth. From the author's experience and from a survey of the literature, the conclusion is arrived at that no one univalent serum will agglutinate all dysentery bacilli of the mannite fermenting type and that a bacillus with the morphological, cultural, and biochemical characters of dysentery bacilli of this type is not discredited as an etiological factor because it is not agglutinated by any particular univalent serum.

In conclusion we express our indebtedness to Miss Rhodes of the Lister Institute for retesting many of the cultures in London, in January, 1917, and convey to her our grateful appreciation of her kindness in so doing.

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THE CONDITIONS OF LIFE IN TROPICAL AUSTRALIA.

By WILLIAM NICOLL, M.A., D.Sc., M.D., D.P.H. (London).

"White Australia," the pleasant dream of a peculiar political party, is a subject on which a few years ago it would have been inadvisable if not unsafe to venture too unrestrained opinions. There was a time when in certain quarters the euphonious phrase was almost tantamount to a war cry. To some political dreamers it may even have been a perennial dream, full of visions of whiteness and purity, just as to us in England the mention of the "Pearly Pacific" conjures up vistas of prattling palms, wimpling waters and silvery strands. The dream is a pretty one, fitting theme for the poet and artist; Samoa and R. L. Stevenson, what a perfect juxtaposition! What delectable tales our romance weavers indulge us with! The gorgeous colouring, the wondrous life, the glorious moon, the ocean's unceasing sigh—all fine, all beautiful, all seeming perfect—but all superficial or tempered with prosaic features which mar the picture.

The Tropics, America, Africa, India and later the Pacific Isles have successively been the "El Dorado" of many a gallant sailor, many a brave soldier and many a specious adventurer. The dreams of untold wealth have been realised full many a time but at what cost it were not wise to think on. The wealth of the Indies connotes something fabulous—but the *health* of the Indies connotes a melancholy fact, known to most, distorted by many.

There was a time, but yesterday it seems, when "the Tropics" was synonymous with ill-health. That truly dreadful term "miasma" was pregnant with significance—mud, vermin, pestilence. A little knowledge, a little haphazardous discovery changed all that and the pendulum of opinion commenced its backward swing. The Tropics again became a possible El Dorado, a happy hunting ground for the ever-adventurous white man. A little white powder became a certain safeguard, so it seemed, and one could visit the Tropics with considerable assurance that no untoward effects would follow. One could indeed venture to remain for months or even a few years.

In spite, however, of the enormous advances in prophylactic medicine and the apparent immunity which they have conferred, there remains the undeniable fact that white people, as such, have never yet succeeded in colonising any part of the Tropics. Exception may be taken to this statement and such instances as the states of Central and Tropical South America be cited. Such instances however illustrate precisely the reverse, for in place of the original European settlers a people or peoples of considerably different characteristics have been evolved. These races have not only diverged more or less widely from the original European stock but have broken up into various individual races presenting differences amongst themselves almost as radical as those separating French and Spanish or English and German. These states represent up till the present the highest level to which European colonisation of the Tropics has reached, and they are assuredly very far removed from the ideals which the exponents of the "White Australia" policy have set themselves.

These particular persons appear oblivious of the fact that change of environment alone will cause radical alteration not perhaps so much in physical characters as in mental habitude. A superficial study of the contrast between the British people and the people of the United States serves to indicate this; a closer study convinces. A century's separation, politically and geographically, has evolved the modern American who, whether superior or inferior to his European prototype, is undoubtedly different mentally. Exactly the same process is taking place in Australia. There are at present very few Australians of the third generation and perhaps not a preponderance even of the second generation. Fully half the population are either Europeans or the children of Europeans. From these it is impossible to draw any conclusion regarding the future. At the same time there can be little or no question that a distinct Australian type is being evolved. The familiar term "cornstalk" as applied to Australians indicates at least one direction in which the change is proceeding.

It is not my intention to enter into any lengthy discussion of the characters which go to individualise the modern Australian. Some of these characters are more or less obvious, as for instance the height, as mentioned above, the build, the voice and so forth. It may be said that some of these characters are merely local and that greater differences are to be met with between say Yorkshiremen and Cornishmen. Whether that be so or not it only serves to illustrate the fact that the differences are due to separation, not necessarily wide but relatively

total. Even at the present day it is not often a difficult matter to differentiate say an educated Scotchman or Irishman from a Londoner. The difference is not betrayed in his appearance, his manner or his speech but in his trend of thought and his mental attitude. Here again it is not a question of superiority or inferiority; it is merely difference.

Very few people will maintain that the difference is hereditary; it is largely or almost entirely local. That it is marked no one can possibly deny. Such being the case it is not surprising to find that a distinctive "race" is being evolved in Australia, not purely British it is true, but very largely so, very much in fact analogous to the American "race." No one at the present day would be foolish enough to speak of Americans as English or British in spite of the fact that until recently a continuous stream of fresh immigration has been pouring into the States from this country. There is absolutely just as little reason or cause to consider the people of Australia or Canada as English, for that they certainly and assuredly are not, physically, mentally or ethically. The modern Australian is a distinctive type, a strong type and a good one but it is assuredly not English. I have already remarked that the physical characteristics are somewhat different; the mental characteristics are decidedly different.

The trend of Australian thought is essentially democratic and materialistic. This is evidenced by the whole social system, political and otherwise.

In literature, in art, in music Australia has evolved types, perhaps not altogether of the highest, but eminently distinctive. The merest glance through the literary product of Australia, not excessively voluminous as yet, cannot fail to convince one that it has a distinctive character which is not entirely due to the local subjects of which it treats. Modern English literature, needless to say, is widely read, but, and this is to some extent indicative, modern American literature is just as widely read, if not more so. As far as one can judge the literary taste does not extend much beyond the novel and lighter shades of poetry and philosophy, but this is perhaps characteristic more of the epoch than of the people.

In Art Australia has advanced much further than in literature and many fine products have been the result of Australian effort in this direction. It is difficult for one possessing little artistic ability and not too much artistic appreciation to estimate correctly the real value of Australian art but that it has attained a moderately high standard is obvious even to the uninitiated.

It is particularly in music both vocal and instrumental that Australia has excelled. The names of many Australian musicians are familiar even in Europe and not a few have reached almost the highest pinnacle of fame. As a people Australians are fond of music and show a full appreciation of its highest expression. This is evident not only in the cities but even far in the heart of the "bush," and a day's arduous journey is often lightly undertaken with the object of listening to some well known musician.

It is thus evident that on an average Australian culture does not reach the level of the older civilization of England but that in certain directions it tends to, if it does not actually, excel it.

In matters scientific, and more particularly medical, Australia is excessively backward. Almost without exception scientific medicine is the prerogative of a relatively few Scotchmen and Englishmen, and indeed until comparatively recently purely Australian science and medicine were practically non-existent. There has been, it is true, of late a tendency towards preferential treatment of the "native" product, but in how far this is successful is not yet evident. A much more reprehensible and indeed almost fatal policy was formerly in evidence, namely the glorification of the scientific foreigner. Even before the war the disaster-laden menace of this policy was only too apparent. The present circumstances in Europe may perhaps do Australian science an enormous amount of good in the future, but one hesitates to be assured in view of the somewhat ignorant—there is no other word—attitude adopted towards scientific men and things scientific.

To arrive at a true conclusion in regard to the matter of Tropical Australia one must consider a variety of opinions and a conglomeration of facts and possibilities, scientific and otherwise. Within the confines of the present paper one can only hope to do so in very brief. The actual country itself must be the first consideration, its position, its character and its possibilities. Even to the most casual observer it must be apparent that Australia is the least favourably situated of all the continents inasmuch as it is the most widely separated from the present centres of civilisation and human progress. Even by the quickest possible route Sydney is at present separated from London by at least twenty days, such a rapid means of transit being available only to a favoured few. To the less favoured a journey of thirty days is necessary. With the present railway and steamship speeds at our command no part of Australia can be reached from London under eighteen days continuous travel, with the longest available stretch of

railway communication (say Calais to Singapore) and the highest available railway speed of sixty miles an hour the journey could not be made under eight days. Thus, under the most favourable present circumstances, Australia can never hope to be as near London as was New York thirty years ago and the enormous difference which these years have made must be sufficiently obvious to most of us.

Under these circumstances it is in the nature of things that Australia must be backward unless, indeed, as might conceivably happen, the centres of civilisation be transferred from the Old World to the New. Even then, however, Australia would still be the most unfavourably situated of all the continents. It is thus evident that for many a long day to come Australia must inevitably bring up the tail of the civilised parts of the earth and must continue to do so until some enormous upheaval, literal or figurative, completely alters present conditions.

The above are facts which even the more intelligent of educated Australians do not fully grasp or realise. The majority of true Australians are proud, and justly so, of their country, of what it has done and what it is doing, but on reflection they cannot fail to be conscious of the enormous handicap under which it suffers. This applies to Sydney and Melbourne, two of the finest cities in the British Empire. It applies with doubled force to Perth, Adelaide and Brisbane. Outside these big centres its application increases by leaps and bounds. In such a place as Townsville, the "capital of the North," a mere village in size, numbering even at the widest computation not more than 20,000 inhabitants, the applicability of the above remarks must be strikingly obvious even to the most casual observer. Yet Townsville is a port which in point of shipping tonnage, entered and cleared, ranks possibly amongst the first dozen in the British Empire, the reason partly being that it is at present practically the only port for an enormous extent of country stretching five or six hundred miles inland to the rich copper mines at Cloncurry.

In the multitude of smaller towns and villages inland one finds a mental attitude much akin to that of rural England, namely a more or less intense concern in local affairs and a somewhat restricted and detached outlook on things beyond, apart that is from purely business matters. At the same time one is certain to find in such communities one or two, perchance a few, men and women of much more than average intelligence and education who, even in their remote obscurity, have not lost touch with the great world and whose knowledge is surprisingly up-to-date. A considerable amount of music and literature

may, in the ordinary course of events, find its way thither and the present war has undoubtedly stirred even these people to a fuller comprehension of external affairs, but science and medicine are as a rule of remote interest. Not a few of these places may be fifty or more miles from the nearest medical aid and many of them indeed illustrate a fact, known to most of us, that human beings can get along very comfortably with very little medical attention except in cases of serious illness or accident. To such people the problems of "White Australia" do not appeal in the abstract but they are ever present in the concrete. While it may be said in a general way that their health conditions are better than those of the coast dwellers, while the climate they enjoy is a more equable and therefore more monotonous one (apart from the extremes of temperature which may differ by as much as 70° or even 80° F.), still they are not exempt from many discomforts and inconveniences. There rain, instead of being a disagreeable nuisance, is looked upon as a life-giver and life-saver. The whole welfare of the district, the life of man and beast is dependent upon the timely arrival of a few rain clouds. Should they fail nothing but ruin, temporary at least, rewards the most patient toil and industry. Such droughts have been not infrequent even in the memory of the present generation, but such is the recuperative power of the virgin country that a year or it may be two suffices to restore it to its former prosperity.

These disastrous droughts will no doubt be of little moment when in the course of time a system of artificial irrigation is introduced more extensively. Even now artesian borings have rendered not a few areas altogether independent of rainfall. Such measures will undoubtedly render large tracts of country perennially productive, but they will do little to ameliorate the climatic conditions and taken by themselves they will tend without fail to render the country somewhat less healthy, for there is not the slightest doubt that the introduction of surface water will attract and encourage the growth of many agents of disease. It is on that account not altogether easy to predict from the present circumstances what the future prospects of Australia will be.

To deal with the question as it is at present one must understand, roughly at least, the configuration of the Australian continent. There is in the first place the coastal belt varying in width from a few miles up to eighty or a hundred. Inland this is delimited by a more or less continuous range of hills beyond which is a flat tableland with a tendency to slope towards the interior. In the coastal belt the conditions are essentially what are generally understood as "tropical," namely, there

is a rainy season usually from November to April and a dry season during the other months of the year. The terms "rainy" and "dry" are not as a rule merely relative; they are absolute. During the dry season there is no rain or at most only unmeasurable quantities at a time; during the rainy season it may rain for days and weeks, even months, with only occasional remissions. In Townsville during 1913–1914 the rainfall only rarely assumed the proverbial tropical type and was usually of a more or less mildly persistent character. Occasionally however especially in the evening there were downpours which could only be described as torrential, usually accompanied by thunder and lightning. These as a rule did not exceed one inch at a time but in certain localities, e.g., Innisfail, the daily fall was to be measured in inches. Innisfail indeed has the reputation of being one of the wettest places in the world, the annual fall there usually exceeding a hundred inches; sometimes attaining five or six times that amount. In Townsville the average rainfall was 40—50 inches, but in 1915, this being a year of drought, it barely exceeded 12 inches. Taking a low average it may with fair certainty be said that the average coastal rainfall of Tropical Queensland considerably exceeds 50 inches.

Inland from the coastal range of hills however the rainfall figure immediately drops. At Charters Towers, which is less than 70 miles inland, the average annual rainfall does not greatly exceed 25 inches, while every mile inland from that sees a further drop. Considerable areas do not receive more than five inches a year and that probably falls in a couple of showers.

There is a corresponding difference in the temperatures. At Townsville for instance the average maximum temperature is about 80° F. During the hot wet season there are days and nights when the temperature remains continuously at or about that figure dropping only a very few degrees during the night. Before the advent of rain the temperature frequently runs well over 90° F., but only occasionally did it exceed 100° F. The minimum temperature recorded during my residence in Townsville was only a few degrees below 50° F. At Charters Towers however temperatures of under 40° F. were comparatively common while in many places temperatures below freezing point are occasionally recorded. Maximum temperatures of over 100° F. are more frequent the further inland one goes.

In the coastal regions, however, it is the humidity of the atmosphere, even when there is no actual rainfall, which is the most trying circumstance. It was a frequent experience to find the wet bulb thermometer

registering only 1° or even ·5° F. below the dry bulb, indicating a very high humidity of the atmosphere.

With regard to the black bulb thermometer, registering the solar radiation, readings of 140° and 150° F. were not infrequent during the hot season, while on very overcast days, the readings not infrequently barely exceeded those of the dry bulb; in other words the sunshine was practically nil.

The local character of the country alters considerably in the change from the dry to the wet season. In the former the predominating feature is sand and dry cheerless vegetation. To keep any plant life alive in the garden an assiduous and constant use of the water-hose is essential. On the other hand as soon as the rainy season sets in thoroughly the ground becomes absolutely sodden. Where once was sandy desert is now lagoon or small pond. The roads once buried in dust become quagmires of soft mud through which course streams of turbid water. The roads and paths leading from the higher lands become the venue of raging torrents over which wooden bridges are a necessity. Needless to say the water plays havoc with the beds of the roads and carries away quantities of stones and earth except on the few roads which have been thoroughly tarred or asphalted. The roads and footpaths in fact become dangerous to unwary pedestrians who require to pick their way with exceeding care.

These torrents and ponds may persist for weeks on end and are reinforced by every fresh shower. It is no uncommon experience for banks of earth to be undermined and carried away and for gardens and yards to be invaded by the floods. The excess of water immediately causes the recrudescence of vegetation luxuriant but rank. This again provides further work for people of gardening propensities.

Mention must be made of one or two other features which may be regarded as amenities or not according to one's point of view. In the first place domestic animals are a nuisance to all who do not keep them. The animals most favoured for domestication are poultry and goats. Swarms of both are to be frequently encountered even within a short radius of the Town Hall. Cattle and horses also roam at large. While confining their attention to waste lands and the outer side of fences they are possibly picturesque, but unfortunately they have a decided predilection for weak spots in fences. A loose spar, a crack or a slight washaway under the fence is discovered with amazing rapidity. This spells rapid destruction for a garden on which perhaps months of patient toil has been expended.

In addition to such common animals as fowls and goats, however, there are several others of a more native character. Perhaps the most destructive and annoying of all are the fruit-bats or flying-foxes, as they are called (*Pteropus gouldi*). During the height of their season they come in countless swarms. Every evening towards sunset a dark cloud begins to gather on the horizon and sails with amazing rapidity across the sky. Presently one becomes aware that the flying foxes have arrived and the air resounds with their raucous screams. Their object is the fruit, principally pawpaws and mangoes which they attack with great avidity. Their squabbling noises frequently resound into the small hours of the morning.

Amongst the native birds which visit the gardens most noticeable are the mynahs, which to some extent take the place of starlings in England, and the extremely graceful "peaceful doves."

Few houses are without their "possums" which frequent the roofs. Their nocturnal noises are rather disconcerting at times as, in moving about, they give one the impression of someone walking through the house. They are however much more tolerable than the swarms of rats and mice which abound in most houses and display a temerity far in excess of that of their English cousins.

The "national" bird of Australia, the kookaburra or laughing jackass, of which there are two species, *Dacelo gigas* and *D. leachii*, is not common so far north as Townsville. Its remarkable vocal effects, however, are very familiar to residents further south.

To my mind one of the most characteristic notes of Australian tropical life is struck by the frogs. Three species occur in great numbers, namely the tree frogs, *Hyla arborea*, *H. gracilenta* and *H. aurea*. Less common but even more characteristic is the burrowing frog, *Chiroleptes brevipalmatus*. These, in addition to birds, constitute the great foes of insect life and are particularly useful in dealing with household insects. They frequent the bathrooms for water and the verandahs for food in the shape of insects, but occasionally they venture within the sacred precincts of the house where their temerity often leads to mishap in the shape of a heavy foot. It is a peculiarly unpleasant sensation to tread upon a frog unawares. There can be no doubt that the presence of these frogs, within reason, is beneficial from a sanitary point of view.

Less welcome but not necessarily harmful visitors are lizards and snakes. Two species of lizards are met with frequently in the vicinity of houses, namely the blue-tongued lizard (*Tiliqua scincoides*) and the

monitor (*Varanus varius*). As a rule they inhabit the spaces under the houses and are not frequently obvious unless looked for. They are probably useful in coping with the insect pests. Of snakes there are several species which are to be met with in or near houses. By far the most common of these is the carpet snake (*Python variegatus*) which is non-poisonous and generally regarded as harmless. It frequently attains a length of 5—6 feet. Other snakes are less frequently seen near human habitations but are met with commonly elsewhere. The most familiar of these is the black tree snake, which has the reputation of being poisonous.

It is when one comes to speak of insects that words fail, for insect life is undoubtedly the greatest bane of the Tropics and Tropical Australia is by no means exempt. It would be impossible to deal adequately with the multitude and variety of even the common insects and I shall confine my remarks to a comparative few which are particularly obnoxious or have a definite relation to disease.

One has some slight satisfaction in saying that the ordinary English house flies or rather their Australian relations are less common than in England, but their place is taken by a myriad other insects whose attentions are even more annoying and decidedly fraught with danger. The biting stable-fly (*Stomoxys calcitrans*) is very common and a constant source of worry to cattle and horses. The formidable marsh flies (*Tabanus* spp.) are also persistent nuisances particularly in uncleared areas.

Mosquitoes head the list of inveterate pests and few people escape their attentions. Most new residents are not only attacked but show very pronounced signs of such attacks in the form of small raised lumps on hands and face and ankles. Even the covered parts of the body are frequently attacked. The favourite sites of attack are the temples, the angle of the jaws, the back of the neck, the backs of the hands and wrists and the ankles, in fact the parts of the body where the skin is stretched most tightly over the subjacent tissues. In most people these bites are intensely irritating and the effect may persist for a considerable time. Not infrequently a slight septic infection may supervene on the bite. After some months or a year's residence most people acquire a certain degree of immunity either to the bites or to their effects. A few fortunate individuals appear to enjoy a natural immunity. The most common domestic mosquitoes are *Stegomyia fasciata* and *Culex fatigans*. Anophelines are much less common, *Anopheles maculipennis* being that most frequently encountered. That some, or even several of these

mosquitoes are disease carriers is unquestionable, but this will be referred to later.

Next in importance, perhaps, are ants. They are the dismay of the housewife. All foodstuffs of every kind have to be most carefully protected from these insects. Meat safes are an absolute necessity and they require to be guarded by ant traps. Kitchen tables and dining room tables, in fact any furniture on which food of any kind is to be laid must be protected in a similar fashion. This is a constant source of tribulation to the conscientious cook, for no matter how careful she be one or other of these ant traps may go faulty and a procession of ants find its way amongst the viands. It is indeed almost impossible to keep ants away from food, for they discover what appear to be practically impassable routes of access. Although however their presence is undesirable they probably do little harm and they afford compensation by acting as scavengers. Cockroaches, though not so numerous as ants, are quite as great a nuisance. They also chiefly frequent the kitchens and dining rooms. In addition to the culinary department, however, cockroaches are fond of frequenting the library and the bookshelves. The bindings of books generally show numerous marks of their attentions. So far as I have observed, only the smaller cockroach, *Periplaneta orientalis* occurs.

Needless to say clothes moths bulk very largely in the household economy. The most careful precautions require to be taken with all clothes, more particularly silk and woollen garments. Heavy silks are not suited to the climatic conditions but lighter silks and mixtures of silk and cotton are frequently used both for dresses and for underwear. They suffer most disastrously. Heavier woollen underwear which is generally packed away during the greater part of the year is frequently found reduced to fragments when necessity arises for its being unearthed. Linen, cotton and tweed generally escape except when stained by food.

Amongst other domestic pests mention must be made of spiders which are fairly common. The most familiar spider is an enormous green one, measuring three inches, which is quite common. It is popularly known as the tarantula, but for what reason I could never discover. So far as I know it is perfectly harmless and indeed useful in some respects. Its size and appearance make it somewhat repulsive, but as a rule little or no notice is taken of it. This is the only domestic spider I have personally seen in the north though several others are to be met with.

With regard to body parasites fleas and lice both occur, but, to

the best of my knowledge, not in excessive numbers. The great ubiquity of rats probably prevents fleas becoming too great a menace to human comfort. *Xenopsylla cheopis*, the Indian rat-flea, is probably the commonest flea but *Pulex irritans* and *Ceratophyllus fasciatus* also occur on rats.

Phthirus pubis is, needless to say, a common human parasite, while *Pediculus capitis* and *Pediculus corporis* are not infrequent, but I have never observed cases of such heavy infestation as are only too frequently seen in this country.

The human diseases of Tropical Australia may be divided into two categories, namely, those which are common in countries outside the Tropics and those which are purely tropical in distribution or almost entirely so.

It must be remembered in the first place that the death-rate in Australia as a whole is extremely low, considerably lower than that of most European countries. In the tropical regions, however, the rate is much higher than in the non-tropical parts, probably on an average being at least half as high again. There are several fairly obvious reasons why the death-rate in Australia in general should be low. In the first place the type of emigrant is fairly good physically. The immigration authorities exercise a certain amount of supervision over persons admitted into the country and any suffering from obviously chronic ailments are rejected and sent back. Moreover persons over a certain age are not admitted except for special reasons. It must be said that the medical examination is not excessively strict but it undoubtedly prevents the dumping of a crowd of undesirables and unfits.

It is thus evident that the class of people who are admitted to Australia are persons for the most part between 18 and 50; persons who are in the prime of life and amongst whom disease incidence and death-rate are considerably lower than amongst the two classes, namely young children and aged, in which these factors are relatively high. The number of infants admitted under one year is comparatively small. These are considerations which must be taken into account in contrasting the death rate of Australia with that of England. When this is done it is at once evident that the difference is not so very greatly in favour of Australia if indeed at all.

Taking into consideration, again, the matter of urban and rural distribution of population, there is a fairly prevalent idea in this country that all Australians live in the bush or scrub or at any rate somewhere in the open country, and that they live a free life bounded only by the

distant horizon. Such a view, needless to say, is hopelessly erroneous. Of the total population of Australia, say 6,000,000, more than one quarter live in five towns. An equal number live in thirty other towns, so that over one half of the population are pure town dwellers. Another half million are crowded into the comparatively small coastal corner of Victoria and New South Wales, so that in the remainder of the vast continent there are not more than 1,500,000 people, i.e., a density of population of one person to every two square miles.

Roughly speaking then it may be said that New South Wales and Victoria are moderately well populated, Queensland and South Australia have about one person per square mile, while West Australia and the Northern Territory are almost uninhabited other than in the small district around Perth. It is thus manifestly impossible to consider conditions in Australia as even approximately homogeneous.

It is not my intention to deal here with the conditions determining the occurrence of disease throughout the whole length and breadth of Australia. Many interesting facts and observations are to be found recorded in the official reports of which perhaps those furnished by the Government Bureau of Microbiology in Sydney are the most instructive. Moreover an extremely useful series of reports on several special diseases has been published by the Department of External Affairs. From a survey and examination of these documents much information may be gathered.

With regard to constitutional diseases in general but little need be said beyond remarking that all the well-known old-world diseases are met with. Cardiovascular and renal diseases are probably quite as common in Australia as they are in Europe and are due to the same causes. Respiratory diseases, particularly tuberculosis, pneumonia and pleurisy are less frequent. This is undoubtedly chiefly due to the fact that conditions of life are at present decidedly better in Australia than in Europe, that there is practically no destitution and very little tendency as yet to overcrowding, except in certain areas of the large cities. Nervous and mental diseases, apart from those of specific origin, are also comparatively uncommon.

Of the specific diseases, syphilis and gonorrhoea are apparently quite as common as they are in England. So far as my knowledge goes neither disease is so virulent or followed by such disastrous effects, as in this country. This is probably is some part due to greater facilities for early treatment and to a better informed public opinion in regard to the diseases. The attempt, indeed, has been made to make these diseases compulsorily notifiable.

It is particularly in the matter of exanthematous infectious diseases that comparison becomes of interest. There can be but little doubt that these diseases afford by far the best indication of the health of a community and of the efficiency of the official and private measures which are taken to preserve health conditions.

Typhoid fever, perhaps more than any other disease, may be taken as an indication of the sanitary cleanliness of any community. It is to be regretted that Australia cannot congratulate itself on an immunity from this disease, of which there has been more than one epidemic within the past ten years. In some areas indeed the disease is apparently endemic, or what is more probable many undetected carriers occur. Little attempt has apparently been made to deal effectively with the carrier problem. The use of typhoid vaccine, however, is gradually being adopted. The paratyphoid fevers are very rarely diagnosed, but in this respect Australia is not much worse than England. That bacillary dysentery occurs there can be little question; that it is rarely diagnosed specifically as such is equally certain. With regard to epidemic diarrhoea of children I have little information, but that it occurs is highly probable.

Of all the intestinal disorders sprue is probably the most characteristically tropical. With regard to its origin and causation we possess little knowledge of value. Some attempt has been made to study the disease in Australia but it has been of a most particularly futile character. Although it is not excessively common in Tropical Australia sprue is undoubtedly one of the most certainly fatal diseases. No treatment has yet been found to give invariably satisfactory results in a well-established case. The disease is almost invariably chronic and progressive.

Of the acute infectious fevers dengue fever is that which has been the cause of more sickness and ill health than any other disease in Tropical Australia. Its occurrence is apparently seasonal but an epidemic may extend over the better half of a year. In a recent epidemic to which I personally fell a victim a very large percentage of the population was attacked. The disease most resembles perhaps influenza of the muscular type. In addition to the fever which is usually fairly abrupt in onset there is usually a distinct prodromal rash of some indefinite type; there are generally pains in the muscles of the back, sometimes in the limbs, and headache and general malaise. In the majority of cases the illness is short and the symptoms mild, but even in such cases the post febrile effects may be serious. The heart is frequently dangerously affected, a common sequel being myocarditis of an acute or chronic type. The individual muscle fibres are damaged and a process of

degeneration follows the acute stage. The valves are not primarily affected but the whole organ loses tone and becomes flabby. Dyspnoea on excretion is the most prominent feature and may persist for many months after the acute symptoms have passed. In my own case the myocardial affection is still evident even two years after the actual fever. It may be of interest to note that the condition was diagnosed as myocardial degeneration by two leading London heart specialists, irrespective of any knowledge of the previous history.

Such an infection with such a sequel can only be expected to have serious consequences in persons over fifty, and there can be little doubt that this has been the cause of death in most of the fatal cases.

Dengue fever was epidemic during two successive years, the epidemics being very widespread. A large part of the population of Townsville and adjoining districts was infected. Numerous instances of rather severe illness were recorded and several deaths occurred. The fever itself is not necessarily of a very intense character, but the after effects are frequently most disastrous. Chief amongst these must be mentioned the profound depression which may occasionally lead to suicide. Short of this there are the above mentioned cardiac affections, chiefly of the myocardium, leading to enfeebled muscle substance ("brown degeneration").

Malaria, the most notorious though not necessarily the most deadly of tropical diseases, is comparatively uncommon in North Queensland. While however it is uncommon in Queensland as compared with other tropical regions still in certain restricted areas it is sufficiently common to cause much apprehension. The majority of the cases originating in Australia are of the benign tertian type, but many serious cases are imported from adjoining regions, e.g., New Guinea.

Plague has not yet obtained a firm foothold in Australia thanks largely on the one hand to more or less efficient quarantine administration and on the other to the sparsity of population. All the other natural factors involved in the spread of the disease, however, are present. Rats are plentiful though not excessively numerous, and they carry with them a sufficient number of fleas to serve as intermediaries. Outbreaks of plague are not unknown in Australia, but they have invariably been kept within moderate limits.

Cholera, so far as one can gather, has not yet found its way into Australia and with reasonable precautions there is no reason why it ever should. Water supplies are, as a rule, of fairly good quality, and sanitary arrangements, though far from perfect, are sufficiently effective to prevent any extensive spread.

Similar comment may be passed in regard to dysentery in its various forms. That bacillary dysentery of undetermined type has occurred in some parts of Northern Australia is fairly certain, but so far as I am aware no record has yet been made of the amoebic form. It is not improbable that the return of Australian troops from Egypt and Mesopotamia may serve to introduce the disease to some considerable extent. The problem of the chronic carrier will have to be faced by the health authorities in Australia as in other countries.

Leprosy, though not necessarily a tropical disease, is most prevalent in hot countries. It is not at all common in Australia. A certain number of cases are to be met with amongst the natives but it is disquieting to find that occasional cases occur amongst the white population. Though not numerous they are regarded as sufficiently dangerous to necessitate segregation.

A febrile condition which is of considerable pathological interest and clinical importance is that to which has been given the name of Mossmann fever or endemic glandular fever. This disease occurs almost exclusively in a small circumscribed coastal area around Mossmann and has been studied most systematically by Dr P. S. Clarke. A more recent and somewhat flamboyant attempt to investigate the disease by Fielding, Breinl and Priestley has added little to our knowledge and less to our means of treatment of the disease.

Amongst other diseases which occasionally come under notice mention may be made of climatic bubo, keratosis, ulcerative granuloma and "barcoo rot." These conditions however are uncommon and little has been done in Townsville towards the amelioration of any of them.

Of more interest and importance is the investigation of ring-worm made by Priestley. The disease is not particularly common and is apparently unusually amenable to treatment.

Of even greater importance is the occurrence of lead poisoning, particularly amongst children. The affection has been known and studied in Australia for over thirty years and the symptoms have been accurately described by several medical practitioners. A biochemical investigation of the matter by W. J. Young provided some further useful information. Although no definite conclusion could be arrived at as to the actual means of infection, the views of earlier observers were accepted, namely that the poisoning is a result of inhalation of dust containing dried lead and that intestinal poisoning is much rarer.

Last, but very far from the least important, of disease-producing agents in Queensland are parasitic worms. Although, in this country,

these are generally regarded as negligible factors in the production of disease their importance is invariably grossly underestimated. Yet there cannot be the slightest shadow of doubt that hookworm disease (ankylostomiasis) is at present one of the greatest scourges of mankind. It yields precedence only to tuberculosis, plague and the malarial fevers. The disease is known to be endemic in every country in the tropical belt, including India and large part of China, and the whole of Central America with the Southern United States. It extends into the temperate zones, but only under exceptional circumstances. Malaysia is highly infected and Northern Australia has not escaped.

The number of cases of hookworm disease which present themselves for medical treatment is as a rule relatively small, but the number of cases of light infection cannot fail to be considerable. It must be remembered however that, as I pointed out some years ago[1], the symptoms are not invariably proportionate to the number of worms present in any particular case.

Beyond treating such cases as do seek treatment little has been done towards remedying the conditions which encourage the spread of the disease. I devoted a considerable amount of time and energy to a study of the conditions influencing the development of the worms, but the results obtained, explicit enough though they be, can only be of value if they are followed not only by efficient sanitary control but also by well advised parental control. The only remedy appears to be to make promiscuous defaecation a serious offence with heavy penalty. It seems unpardonable that a disease which can be so easily prevented should be allowed to play havoc with so many school children. Westphalia and the Southern United States ought to be at once a warning and an example to Tropical Australia.

While ankylostomiasis is by far the most serious worm infection met with in Queensland it is by no means the only one. Another worm which appears to give rise to serious symptoms is *Strongyloides intestinalis*. It is frequently found associated with, but not so common as, *Ankylostoma*. It generally gives rise to persistent diarrhoea. Another worm which is comparatively common is *Trichuris trichiura* (*Trichocephalus dispar*). Though generally regarded as harmless this worm undoubtedly gives rise to symptoms which indicate intestinal irritation and malnutrition. *Oxyuris vermicularis* does not appear to be so common as one might have expected, or possibly when occurring alone it does not give rise to symptoms sufficiently urgent to call for medical attention. The

[1] *The Blood volume in Ankylostomiasis.*

majority of cases which have come under my notice have been cases associated with hookworms.

One of the most important of worm infections which occur in Queensland is Filariasis. It is very widespread and appears to be almost as frequent in Southern Queensland as in the tropical parts. The most serious manifestations of this infection are lymph scrotum and elephantiasis. Short of surgical treatment nothing is of any benefit in the former affection while in the latter even surgical treatment cannot be expected to effect a radical cure.

It was found that about 17 per cent. of all patients admitted to the Brisbane General Hospital during 1910 were infected with *Filaria*. This percentage is almost certain to be considerably higher in Northern Queensland, though according to Breinl only 3·4 per cent. of the patients admitted to the Townsville General Hospital are carriers of *Filaria*. Even taking this low estimate we must conclude that there are at least 20,000 people in Queensland who are infected. In view of the serious consequences of the disease this is a condition of affairs which must be viewed with the greatest apprehension.

Tapeworms do not appear to be of frequent occurrence. I possess records of only three cases in Townsville and in every instance there was a strong possibility that the infection had been acquired outside Australia.

So far as I am aware liver flukes or Trematode parasites have not yet been met with amongst the white residents of Tropical Queensland, though a few cases have been recorded from Chinamen in the Northern Territory. These were *Clonorchis endemicus*, a common parasite in China: *Schistosomum haematobium* has been reported in West Australia, but no cases have been recorded in Queensland. In one case some specimens were submitted to me as such but they proved to be merely shreds of fibrous tissue.

There remain a few diseases of which some mention may be made, not because they are common in Tropical Queensland but because there is a possibility of them becoming more widespread. Beri-beri is largely a disease of rice-eating peoples. A certain number of cases have been observed in Australia, but as it is a disease which rarely attacks white people its importance is not particularly great.

Gangosa is another disease the etiology of which still remains obscure. It takes the form of an ulcerative state of the nose and mouth which is chronic and progressive. It is fairly frequent in New Guinea, the Philippines and one or two other groups of islands. It bears, in some cases, a superficial resemblance to leprosy.

It is not my intention to enter largely here into the vexed question of the influence of climate, as such, on white people. In making any study of this matter one is confronted at the outset with a conflicting variety of intercurrent circumstances, which baffle individual determination. Briefly the matter resolves itself into the question, "Is a tropical climate of itself inimical to the health of European people?" The answer can be one only, "We do not know." I have already remarked that Australia is evolving a distinctive race with several well marked characteristics. These however are common to the country as a whole and not to any one part. It remains to be seen whether that race will be able to colonise the Tropics any more successfully than purely European races. Personally I am extremely doubtful, for it is a matter for common comment that Australians from the South are as much distressed by tropical conditions as are fresh arrivals from Europe, if indeed not more so. It is at any rate a fairly general practice amongst the larger business firms to transfer their more highly placed employees to more southern billets after a longer or shorter residence in the Tropics. It is in fact a generally understood agreement that an employee will sooner or later be transferred unless he be condemned to remain, as a punishment for his "shortcomings."

It may be maintained that this inability or unwillingness to remain in the tropics is largely a mental disability. Such a contention means nothing, for mental disability is quite as serious a matter as physical disability or ill-health. There is not the slightest doubt that residence in the remote tropics causes much mental stasis which to those trained in mental rather than manual labour, is quite as prejudicial as stasis in any other vital function. This is both a direct and an indirect result of tropical conditions.

There is however the possibly more important question of actual physical change in the anatomy and more particularly the physiology of white persons resident in the Tropics. This question has given rise to considerable speculation and not a little actual research. It is a matter which has engaged attention in several quarters for a number of years, and it cannot be denied that some interesting facts have been brought to light. Changes in the various organs and structures of the body have been noted, but in no instance does it appear that these observations have been sufficiently extensive or free from error to form the basis for any unequivocal judgment.

In Queensland an ambitious scheme was entered upon for the examination of school children. It was far from original and further from

efficient. Blood examinations were made on a few hundred children and on a few dozen Papuan natives. Although taking no immediate part in these investigations I had frequent opportunity of observing the methods of procedure, methods which to one accustomed to accurate haematological work made the blood run cold. Counts and estimations were nevertheless made and calculations and charts in due course evolved from these. As neither collaborator apparently possessed any but the most elementary knowledge of mathematics or statistics it is not to be wondered at that, as an *interpretation* of the facts of the case, the results are absolutely and unequivocally worthless. Results they certainly are such as the merest schoolboy with slate and pencil might laboriously evolve. Results of any statistical value they most assuredly are not. It is possible that in the hands of an expert statistician the figures might be put to some use. As they stand they can only be regarded as an example of assiduity combined with ignorance. The principal result obtained was that in the "Arneth index" of the polymorphonuclear leucocytes there was a decided "shift to the left."

Interpreted in plain language the foregoing means that in European children born in the Tropics there is a relatively higher proportion of polymorphonuclear leucocytes with two or three nuclear lobes than with four or five. What the precise significance of the number of lobes in a polymorphonucleate leucocyte may be is still a highly debateable question and any result based upon this is manifestly equally debateable. On that account we cannot as yet congratulate the Townsville experts on their results, more especially as we unfortunately have seen these results "in the making."

As specimens of what sententious balderdash may be expressed on this subject, some remarks may be quoted from the concluding sentences of Breinl's lecture on the "Influence of climate, etc., on the white race living in the Tropics." He says, "The European with energy and ambitions will, as a rule, be only slightly affected by the changed conditions of life and the alteration of his social condition. Even he will lose a certain amount of his energy; he will feel tempted to succumb to the fascination of the *dolce far niente*. A call on his energy will, however, always be answered and he will be able to do nearly the same amount of work bodily and mentally as anywhere in Europe."

These three innocently pious expressions of opinion are mutually and hopelessly contradictory and are at the same time destructive of the great aim and object of the "White Australia" policy. In the first place the "European with energy and ambitions will, as a rule," *not*

"be only slightly affected, etc." He will not be affected *at all*, for the very simple reason that he will not be foolish enough to bury his energy and ambition in such a forsaken corner of creation as North Queensland. "The European with energy and ambition will as a rule" find ample scope for the display and exercise of his talents somewhere in Europe. We cannot conceive that permanent residence in North Queensland can be a sufficiently attractive goal for any "European with ambition." It might possibly form a stepping stone; never a goal.

As for the aforementioned ideal European losing "a certain amount of his energy," nothing could be more certain. A month's continuous hot weather in England with a comparatively low humidity tries the endurance of even the most phlegmatic and industrious of men. Double the relative humidity or even increase it by half, give them a six months' spell of it, repeat the process every year for five years, and then make tactful enquiries as to their ambitions. Cut off at the same time their refreshing week ends out of town, their various exercises and amusements, and watch the result. It might result in increased attention to business or the reverse, either of which is the initial step in a particularly vicious circle.

I have met many people in all walks of life in Queensland but I have met few who were enamoured of the climate of its coastal parts. Even the author of the above quoted sentences is guilty of a slight misrepresentation if he makes pretence that they represent his own unqualified views on the matter at the time they were written.

In conclusion, then, I may venture, with due reserve, to give it as my opinion that Tropical Australia will never *under present circumstances* support a permanent population of *exclusively* European character. That it will support a population of European descent I have no doubt, but it will be little more European than are the peoples of the Central and South American States.

To my mind the chief hope of permanently establishing a white population in Tropical Australia lies in a system of residence for a definite number of years, not more than twelve or fifteen, with a definite guarantee of an equivalent position in a temperate region at the end of that service. A liberal vacation should also be allowed at least every second year during the hot season. Such a system is in vogue at present with several of the larger business firms and appears to be satisfactory. Amongst the employees of these firms are to be found the keenest and most enterprising men in the Tropics. Were this enterprise and assiduity to be rewarded only by prolonged exile in the Tropics they

would without the slightest doubt rapidly reach a lamentably low ebb.

Such a system would benefit not only the individual but the business firm as well. The enterprising man would find plenty of scope for his activity and he would be constantly buoyed up by the thought that he was working towards an end which would benefit himself by, amongst other things, securing his transfer to a more congenial locality. This system would have the additional advantage of giving a much larger number of men an opportunity of experiencing tropical conditions and possibly some, if not many, might be found who preferred tropical conditions to those of a temperate clime. Such would undoubtedly form a much more promising nucleus for a white tropical population than men who were forced to remain under tropical conditions against their desire and to the detriment of their health and character.

To accomplish this end what are really needed are men of the widest sympathy and experience, men to whom the future and the development of Australia mean something very real, not mere adventurers whose main object is exploitation and personal aggrandisement.

A STUDY OF THE COLIFORM ORGANISMS INFECTING THE WOUNDS OF WAR.

By MATTHEW J. STEWART, Captain, R.A.M.C. (T.),
Pathologist, East Leeds War Hospital.

(*Report to the Medical Research Committee.*)

(With 1 Chart.)

Already during the present war an extensive literature has grown up on the subject of the bacteriology of septic wounds. It was thought that a detailed study of the coliform organisms met with in these wounds might yield results of interest, and be a useful addition to some of the more general bacteriological contributions.

Cases Investigated.

The material for bacteriological investigation was obtained from the unhealed wounds of soldiers admitted to the East Leeds War Hospital during the second half of 1915 and first half of 1916. In most cases cultures were taken within a few hours of the patient's admission to hospital, but sometimes not for a number of days or even weeks thereafter. The cultures were taken quite indiscriminately from all sorts of wounds, from those which appeared clean as well as from those which were obviously septic, but the great majority belonged to the latter class. Sloughing of bone or of the soft tissues was present in many of them. One wound only was examined in each case, and where more than one wound was present the most septic was selected for investigation. 122 wounds in all were investigated for organisms of the "coliform" group, and of these 70, or 57 %, gave a positive result. Fleming (1915), working at a base hospital in France, found organisms of the coliform group in 74 out of 210 wounds examined, i.e. 35 %. Dean and Mouat (1916), in the investigation of eighteen gangrenous wounds at a home hospital, isolated coliform bacilli in four cases, or 22 %. Dudgeon, Gardner and Bawtree (1915), working in London, found coliform bacilli in not more than 37 wounds out of 100 examined.

In positive cases the length of time intervening between the receipt of the wound and the taking of the culture varied very greatly, the shortest period being five days, the longest 350. It was found, however, that coliform organisms occurred with considerably greater frequency in old wounds than in those which were recently received, and this is particularly true of *B. pyocyaneus*. In the negative cases the average interval between wounding and culturing was 26 days, in the positive cases 49 days, where *B. pyocyaneus* was present 64 days. A more truthful representation of these facts may be made by dividing up the cases into groups corresponding to the stages suggested by Fleming (1915) (Table I).

TABLE I.

To show proportion of wounds, at different stages, infected by coliform bacilli.

Time after infection	No. of cases investigated	No. infected with coliform bacilli	Percentage of positives
Stage I 1–7 days	17	7	41
Stage II 8–20 days	47	20	42
Stage III Over 20 days	58	43	74
Totals	122	70	57 % (average)

If these results are compared with Fleming's, a striking similarity is observable (Table II). The discrepancy in the total percentage is of course accounted for by the higher proportion of cases investigated by Fleming in the earlier stages, when the percentage of positives is lower. The generally higher percentage of positives in my series at all three stages may be partly accounted for by the greater opportunity for contamination afforded by the more prolonged transport.

TABLE II. (Modified from Fleming.)

For comparison with Table I.

Time after infection	No. of cases investigated	No. infected with coliform bacilli	Percentage of positives
Stage I 1–7 days	127	37	29
Stage II 8–20 days	56	18	32
Stage III Over 20 days	27	19	70
Totals	210	74	35 % (average)

Methods.

The purulent discharge from the wound or a scraping from the surface, if the wound were a clean one, was plated directly on Grünbaum and Hume's (1902) modification of MacConkey's medium (neutral red, crystal violet, bile salt, lactose agar), and incubated for 24 hours at 37·5° C. Discrete colonies of about 1 millimetre diameter and upwards were then picked off and subcultured on bullock's heart agar (Douglas, 1914) for a further 24 hours. The colonies on the bile salt plate were first carefully scrutinised through a hand lens, and as far as possible only one colony of each kind present was subcultured. It was found that by the use of Grünbaum and Hume's medium a great variety of appearances by different coliforms was obtainable, a much greater variety than by the use of the neutral red, bile salt, lactose agar of MacConkey. Often one or more subcultures on bile salt plates were made before finally subculturing on agar, especially if there was any difficulty in obtaining discrete colonies of individual bacilli. After confirmation of the morphological and tinctorial characters of the organism from the agar culture, except in the case of *B. pyocyaneus,* all or most of the following media were inoculated, viz. taurocholate broth containing the following 20 substances, glucose, lactose, saccharose, dulcite, mannite, adonite, inulin, salicin, sorbite, laevulose, galactose, raffinose, maltose, arabinose, dextrin, isodulcite, inosite, glycerin, amygdalin and erythrite, litmus milk, gelatin stabs, gelatin slopes (for motility), peptone water (for indol formation), glucose peptone water (for Voges and Proskauer's reaction), and nutrient broth. In the case of the carbohydrate media and the litmus milk the method of massive inoculation by large loopfuls of culture was practised, and it was found that by using large slopes of bullock's heart agar an abundant growth for this purpose was obtainable within 24 hours. Acid fuchsin (Holman, 1915) was used in the sugar broth tubes as colour indicator and proved most satisfactory. This medium, in the absence of acid, is straw coloured. As soon as acid is formed the colour changes to varying shades of red, from pale rose pink to bright carmine, according to the degree of acidity. A notable advantage of this method is that the results are easily legible by artificial light. The gelatin cultures were grown first at 22° C., then at room temperature, all the others at 37·5° C. In the case of the carbohydrate media the results obtained were noted every day for a week, then again at the end of a fortnight, after which they were discarded. The litmus milk tubes were also observed daily for the first week, but as a rule they

were retained for three or four weeks before being finally discarded. The gelatin stab cultures were kept in the 22° incubator for several weeks and the results noted from time to time. After that they were kept at room temperature for many months, never less than six except where complete liquefaction had occurred.

The Voges and Proskauer reaction was carried out in the usual way by adding an equal quantity of 2 % caustic soda to a three days glucose-peptone-water[1] culture of the microbe, and allowing the test tube to stand on the bench for two or three days. In positive cases a pale orange or pinkish colour with greenish fluorescence makes its appearance in a few hours, and is usually well marked by the following day. The appearance has been aptly likened to that of much diluted alcoholic eosin.

The functions of indol formation and of motility were investigated at some length and may be referred to in greater detail.

Indol Formation.

The indol forming powers of the bacteria were studied by the application of Ehrlich's rosindol reaction. After some preliminary experiments, the following procedure was adopted for routine examinations. The organism to be studied is subcultured from a recent agar slope to a tube containing about 5 c.c. of peptone water[2], and incubated for seven days at 37·5° C. A ring test (Tobey, 1908) is then carried out by floating 1 c.c. of Boehme's reagent[3] on top of the culture by means of a pipette. A positive result is indicated by the gradual development of a rose red or pink ring of varying intensity at the line of junction. Usually one or two minutes suffice for the full development of the reaction. Confirmation of the result is obtained by shaking up the contents of the test tube with 1 c.c. of amyl alcohol, as recommended by MacConkey (1909). In positive cases the alcohol extracts the pigment (rosindol), and forms a pinkish layer on the surface. The test tube (uncorked) is then allowed to stand on the laboratory bench for some days, when the

[1] Peptone (Witte) ... 10 grm.
Glucose ... 20 grm.
Sodium chloride ... 5 grm.
Water ... ... to 1000 c.c.

[2] Peptone (Witte) ... 10 grm.
Sodium chloride ... 5 grm.
Water ... ... to 1000 c.c.

[3] Paradimethylamidobenzaldehyde ... 4 grm.
Absolute alcohol (96 %) ... ... 380 c.c.
Concentrated hydrochloric acid ... 80 c.c.

colour of the amyl alcohol slowly deepens to a bright cherry red. In negative cases the colour of the alcohol ranges from grey to varying shades of green and yellow. I have carried out this test in the case of several hundred coliform organisms, and in every instance a definitely positive or definitely negative result has been obtained; there have been no indeterminate results. It has also been shown in the cases examined that the ring test by itself yields thoroughly reliable results; in every case the extraction by amyl alcohol has confirmed the original finding. The addition of an oxidising agent, for example, potassium persulphate, has been found to be unnecessary either for the ring test or for the subsequent extraction. Some interesting observations have been made as a result of keeping the test tubes for some time after amyl alcohol extraction. At first, after the culture plus the benzaldehyde has been shaken up with the alcohol, the latter in positive cases shows a pale pink tint on rising to the surface. This coloration, however, disappears in the course of an hour or two, but after one or two days it returns and gradually increases in intensity. After standing for a week or ten days on the laboratory bench, all the positive cultures show a bright cherry red to deep carmine coloration of the alcohol, which slowly deepens. At the end of a month or six weeks, and still more at the end of two months, the subjacent watery medium assumes, in positive cases, a fairly intense pinkish-violet tint, whereas in negative cases it gradually passes from a greyish or yellowish colour to a pronounced slate blue. Similar phenomena have been observed by MacConkey (1909) and by Seidelin and Lewis (1911). The presence of the blue coloration has been shown by Kligler (1914) to be due to some substance in the peptone and to be entirely independent of the indol test.

MOTILITY.

A large number of experiments were carried out to determine, if possible, the best conditions for the development of motility. The media used were agar (slope, and water of condensation), gelatin, nutrient broth and peptone water, the gelatin being incubated at 22° C., the others at 37·5° C. It was found that on the whole motility could be best demonstrated in an 18 to 24 hours gelatin slope culture, but that a six hours nutrient broth culture was almost as good. Peptone water and the water of condensation in an agar slope were less reliable, the surface of an agar slope notoriously so. The most important point determined was that no one method was infallible. In some cases motility was demonstrated in gelatin cultures when broth cultures failed

to show it, and vice versa. It may therefore be stated that while motility once demonstrated is conclusive, its apparent absence cannot be accepted as final.

CLASSIFICATION.

In deciding which organisms should come within the purview of this investigation the term "coliform" has been interpreted in a very broad and inclusive sense. It has been taken to include all the small, gram-negative, non-sporing bacilli which grow readily on bile salt media and which give a more or less abundant growth on agar. This arbitrary classification has been found to work well in practice, and it is usually quite obvious from the characters of the colony on bile salt agar whether a given organism should be included or not.

Altogether 148 organisms from 70 wounds have been studied, all duplicates from a single wound being excluded. 24 of these are the *B. pyocyaneus*. Of the remaining 124, 86 fall into four well recognised groups, 34 into two atypical but fairly well defined groups, while four only are unclassified. The total number of varieties is 53 (see Table XIII, p. 314). It will be well to give, in the first place, the salient features which have determined the place of each organism in the classification scheme adopted.

(1) *B. coli group.*

Fermentation of glucose and lactose with or without the formation of gas.

(2) *B. proteus group.*

(*a*) Fermentation of glucose and saccharose with formation of acid and gas.

(*b*) Non-fermentation of lactose.

(*c*) Rapid liquefaction of gelatin.

(*d*) Clotting and bleaching of litmus milk and finally more or less digestion of the clot.

(3) *B. Morgan No. 1 group.*

(*a*) Fermentation of glucose, laevulose and galactose only with formation of acid and gas.

(*b*) Formation of indol.

(4) *B. faecalis alkaligenes group* (one strain only obtained).

(*a*) Fermentation of none of the carbohydrates tested.

(*b*) Motility present.

(*c*) Litmus milk rendered strongly alkaline.

(*d*) Gelatin not liquefied.

(5) *Group X.*

(*a*) Fermentation of glucose, laevulose, galactose and inosite, with formation of acid but no gas.
(*b*) Non-fermentation of lactose.
(*c*) Litmus milk rendered acid and then strongly alkaline.
(*d*) Motility present.
(*e*) Formation of indol.

(6) *Group Y.*

(*a*) Fermentation of galactose without formation of gas.
(*b*) Non-fermentation of laevulose.
(*c*) Motility absent.

(7) *B. pyocyaneus.*

(8) *Unclassified.*

Table III is a list of the positive cases, showing (1) the situation of the wound, (2) its duration at the time of examination, and (3) the coliform bacilli present arranged in the above-mentioned groups. Compound fractures when present are indicated in column 2. It will be observed that different cases varied greatly as to the number of varieties which they harboured. Thus from 24 cases one variety of coliform only was obtained, from 26 cases two varieties, from 15 three, from 2 four, from 1 five and from 2 seven. The 46 cases from which two or more varieties were isolated include 11 in which a second examination of the wound was made, usually several weeks after the first. As a rule the wounds which contained a number of varieties were highly septic and often stinking, but not necessarily of long standing. It was not found that any particular variety preponderated in the early stages. Thus from a wound five days old an organism belonging to Group Y was isolated, from another five day wound two varieties of *B. coli*, from a six day wound a coli, a proteus, and a member of Group Y, from a seven day wound a member of Group X, etc., etc. The only organism exhibiting a marked preference for any particular stage was *B. pyocyaneus*, which, as already indicated, tended to occur late. The earliest dates at which it was found were 14 and 17 days. (The distribution of the various bacterial groups is further referred to under "General Remarks.")

Table III.

List of positive cases with coliform bacilli arranged in groups.

No.	Site of wound, etc.	Duration of wounds (days)	*B. coli* group I	II	III	IV	*B. proteus*	*B. Morgan* No. 1	*B. faecalis alk.*	Group X	Group Y	*B. pyocyaneus*	Unclassified
1	Thigh	83	.	.	2	.	1	.	.	.	.	1	.
		208	.	.	.	.	.	.	.	1	.	.	.
2	Leg, compound fracture of tibia ...	59	.	.	.	.	.	.	.	.	1	1	.
3	Leg, compound fracture of tibia ...	7	.	.	2	1	.	1	.	.	.	.	.
		54	.	1	.	1	.	1	.	.	.	1	.
4	Pelvis, compound fracture	8	.	.	.	.	.	.	1	2	.	.	.
		78	.	.	.	.	.	.	.	1	.	.	.
5	Calf	9	.	.	1	.	.	1	.	.	.	.	.
6	Thigh, compound fracture of femur	13	.	.	.	.	1	.	.	.	1	.	.
		54	.	.	.	.	.	.	.	.	1	.	.
7	Forearm, fracture of ulna	6	1	.	.	.	1	.	.	.	1	.	.
8	Loin	21	1	.	1	.	.	.	.	.	.	.	.
9	Recurrent sepsis of wrist	162	.	.	.	.	1	.	.	.	.	.	.
10	Forearm	35	.	.	.	.	.	1	.	.	1	1	.
11	Chest, compound fracture of rib ...	40	.	.	.	.	1	.	.	.	1	.	.
12	Back	32	.	1	1	.	.	.	.	.	.	.	.
13	Shoulder	40	.	1	.	.	.	1	.	.	.	1	.
14	Ankle, sinus	48	.	.	.	.	.	.	.	.	1	1	.
15	Lumbar region, sinus	43	.	.	.	.	1	1	.	.	.	1	.
16	Buttock	47	1	.	.	.	.	1	.	.	.	.	.
17	Leg	48	.	.	.	.	1	.	.	.	.	1	.
18	Leg	52	.	.	.	1	.	.	.	.	1	1	.
19	Thigh, compound fracture of femur	40	.	.	.	.	.	.	.	.	1	.	.
		50	.	.	.	1	.	.	.	.	1	.	.
20	Elbow, fracture of olecranon ...	15	.	.	.	.	1	.	.	.	.	.	.
21	Arm	38	.	.	.	.	.	.	.	.	1	.	.
		82	.	.	.	.	.	.	.	1	1	1	.
22	Leg	17	.	.	.	.	.	.	.	.	1	1	.
23	Pelvis, compound fracture	67	1	.	1	.	.	.	.	.	.	.	.
24	Thigh, compound fracture of femur	66	.	.	1	.	.	.	.	1	.	1	.
25	Amputation stump of leg	42	.	.	.	.	1	.	.	1	.	.	1
26	Shoulder	70	.	.	.	.	1	.	.	.	.	.	.
		140	.	.	.	.	.	.	.	.	.	1	.
27	Leg	14	.	.	.	.	1	.	.	.	.	1	.
28	Shoulder	7	.	.	.	.	1	.	.	1	.	.	.
29	Toe, amputated	7	1	.	.	.	1	.	.	.	1	.	.
30	Forearm, compound fracture of radius	19	.	.	.	.	1	.	.	.	.	.	.
31	Knee	46	.	1	.	.	.	.	.	.	.	1	.
		66	.	.	.	.	.	.	.	.	.	1	1
32	Buttock	16	.	.	.	.	.	.	.	.	1	.	.
33	Thigh	17	1	.	.	.	.	.	.	.	.	.	.

Table III (*cont.*)

No.	Site of wound, etc.	Duration of wounds (days)	*B. coli* group I	II	III	IV	*B. proteus*	*B. Morgan* No. 1	*B. faecalis alk.*	Group X	Group Y	*B. pyocyaneus*	Unclassified
34	Leg	38	.	.	.	.	.	.	.	.	1	1	.
35	Hand	37	.	.	.	.	.	.	.	.	1	1	.
36	Leg, compound fracture of tibia and fibula	108	.	.	.	.	1	.	.	.	.	.	.
37	Leg, compound fracture of tibia and fibula	160	.	1	.	2	.	1	.	.	.	1	1
		181	.	.	.	.	.	.	.	.	1	1	.
38	Arm	8	.	.	1	.	.	.	.	.	.	.	.
39	Forearm	5	.	.	.	2	.	.	.	.	.	.	.
40	Leg	12	.	.	1	.	.	.	.	.	.	.	.
		41	.	.	.	.	.	.	.	.	1	.	.
41	Buttock	7	.	.	.	1	.	.	.	.	.	.	.
42	Elbow, compound fracture-dislocation	5	.	.	.	.	.	.	.	.	1	.	.
		34	.	.	.	1	1	.	.	.	.	.	.
43	Buttock	11	.	.	1	.	1	.	.	.	1	.	.
44	Forearm	9	.	.	.	2	.	.	.	.	.	.	.
45	Wrist	8	.	.	1	1	.	.	.	.	.	.	.
46	Leg	24	.	.	.	.	.	.	.	.	1	.	.
47	Face	70	.	1	.	.	1	.	.	.	1	.	.
48	Shoulder	10	.	.	.	.	.	.	.	.	1	.	.
49	Chest	172	.	.	.	.	1	.	.	.	.	.	.
50	Buttock, sinus	350	.	.	2	1	.	.	.	.	.	.	.
51	Thigh	9	.	.	.	.	.	.	.	.	.	.	1
52	Knee	28	.	.	.	.	1	.	.	.	1	.	.
53	Foot	26	.	.	.	1	1	.	.	.	.	.	.
54	Knee	10	.	.	1	1	.	.	.	.	.	.	.
55	Calf, compound fracture of tibia and fibula	34	.	.	.	.	1	.	.	.	.	1	.
56	Calf, compound fracture of tibia and fibula	80	.	.	.	.	1	.	.	.	.	.	.
57	Hand	23	.	.	.	.	1	.	.	.	.	1	.
58	Leg	18	1	.	.	.	.	.	.	.	.	.	.
59	Shoulder	15	.	.	.	1	.	.	.	.	.	.	.
60	Knee, joint involved...	37	.	.	.	.	1	.	.	.	.	.	.
61	Knee	17	.	.	.	1	.	.	.	.	.	.	.
62	Thigh, knee joint involved	25	.	.	.	.	1	.	.	.	.	.	.
63	Leg	32	.	.	.	.	1	.	.	.	.	.	.
64	Buttock	33	.	.	2	.	1	.	.	.	.	.	.
65	Buttock	33	.	.	.	.	1	.	.	.	1	.	.
66	Heel	38	.	.	.	.	.	.	.	.	1	.	.
67	Leg	22	.	.	.	.	.	.	.	.	.	1	.
68	Leg	60	.	.	.	.	.	.	.	.	.	1	.
69	Forearm	48	.	.	.	.	.	.	.	.	.	1	.
70	Knee	84	.	.	.	.	.	.	.	.	.	1	.

B. coli Group.

This group includes all the organisms which ferment glucose and lactose whether they form gas on these media or not, and irrespective of their action on gelatin. It will be shown later that the organisms in this series which ferment lactose and at the same time liquefy gelatin, are more closely related in their other biological reactions to *B. coli* than to *B. proteus*, and they have therefore been included in the *B. coli* group. Altogether 49 lactose fermenters have been isolated from 32 cases, giving a case incidence of *B. coli* infection of 26 %. By utilising the large series of biological reactions already referred to, it is found that this group of 49 organisms can be differentiated into no fewer than 34 distinct varieties (see Table XIII). The group has been divided into four subgroups, as suggested by MacConkey (1905), according to the fermentative reactions with saccharose and dulcite, and the number of strains and number of varieties met with in each are shown hereunder (Table IV).

Table IV.

Subgroup distribution of members of the B. coli *group obtained from wounds.*

	No of strains	No. of varieties
Subgroup 1 (saccharose – dulcite –)	7	5
,, 2 ,, – ,, +	6	4
,, 3 ,, + ,, +	18	12
,, 4 ,, + ,, –	18	13
Totals	49	34

The common characters of the whole group are these:

(1) Fermentation of glucose, lactose, mannite, laevulose, galactose, maltose and arabinose[1].

(2) Non-fermentation of erythrite[2].

(3) Formation of acid and clot on litmus milk.

All other reactions are variable, as shown in the following table (Table V).

A study of this table brings out a number of interesting points. The large number of common characters possessed by the various members of each of the two first subgroups is striking, as is also the general resemblance, in this respect, between the two. The members of subgroups 3 and 4 show much greater variability amongst themselves, while

[1] Only 20 varieties out of 34 were tested.

[2] Only 22 varieties out of 34 were tested.

Table V.

Variable Reactions of the B. coli *group.*

	Saccharose		Dulcite		Adonite		Inulin		Salicin		Sorbite		Raffinose		Dextrin	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Sub-group 1. (5 varieties)	0	5	0	5	0	5	0	5	3	2	5	0	0	2	4	1
Sub-group 2. (4 varieties)	0	4	4	0	0	4	0	4	2	2	4	0	0	1	3	1
Sub-group 3. (12 varieties)	12	0	12	0	2	10	1	11	7	5	9	3	1	0	10	2
Sub-group 4. (13 varieties)	13	0	0	13	5	8	0	13	13	0	11	2	5	2	13	0
Totals	25	9	16	18	7	27	1	33	25	9	29	5	6	5	30	4

	Isodulcite		Inosite		Glycerin		Amygdalin		Gelatin		Motility		Indol		Voges and Proskauer	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Sub-group 1. (5 varieties)	2	0	0	3	5	0	0	2	0	5	2	3	4	1	0	4
Sub-group 2. (4 varieties)	3	0	0	3	4	0	0	1	0	4	2	2	3	1	0	3
Sub-group 3. (12 varieties)	6	0	0	4	11	1	1	0	2	10	7	5	9	3	3	7
Sub-group 4. (13 varieties)	8	3	2	5	8	5	3	3	8	5	8	5	3	10	10	3
Totals	19	3	2	15	28	6	4	6	10	24	19	15	19	15	13	17

the only character common to both subgroups is saccharose fermentation. Subgroups 2 and 3 present certain resemblances, but subgroups 2 and 4 have nothing in common save the group characteristics. Subgroup 3 seems to occupy an intermediate position to 2 and 4, particularly with regard to gelatin liquefaction and the Voges and Proskauer reaction.

The question of the inclusion of gelatin liquefiers in the *B. coli* group is debateable. Perhaps it would be an advantage to recognise a group (lactose +, gelatin +) intermediate between the *B. coli* group (lactose +, gelatin −) on the one hand and the *B. proteus* group (lactose −, gelatin +) on the other. Short of this I am inclined (at least so far as the present series is concerned) rather to include them in the *B. coli* group, with which, in addition to lactose fermentation, they have in common the power of fermenting mannite and arabinose. It will be observed that all the gelatin liquefying lactose fermenters fall into MacConkey's subgroups 3 and 4, i.e. they are all saccharose fermenters, and in this respect they resemble also *B. proteus*. In order therefore to make further comparison of the lactose + gelatin + organisms with the non-liquefying members of the *coli* group it seems reasonable to limit the

survey to subgroups 3 and 4. It is then found that fermentation of salicin and the Voges and Proskauer reaction afford further evidence of the closer affinity of the gelatin liquefying lactose fermenters to the *B. coli* than to the *B. proteus* group (see Table VI). With regard to the action on litmus milk, also, these organisms behave as *B. coli* and not as *B. proteus* (Table XIII).

Table VI.

Showing the affinities of the Gelatin + Lactose + coliforms.

	Salicin +	Salicin −	Voges and Proskauer +	Voges and Proskauer −
Gelatin − members of subgroups 3 and 4 of *coli* group	10	5	5	8
Gelatin + ,, ,, ,,	10	−	8	2
B. proteus group	−	4	−	4

Indol formation by the B. coli *group.* Of the 34 varieties studied 19 formed indol on peptone water, 15 did not. Indol formers markedly predominate in subgroups 1, 2 and 3; the reverse is the case in subgroup 4 (Table VII).

Table VII.

Formation of Indol by the B. coli *group.*

	Total No. of cases	No. of cases indol +	Percentage of indol + cases
Subgroup 1	5	4	80
,, 2	4	3	75
,, 3	12	9	75
,, 4	13	3	23

Voges and Proskauer's reaction. In the present series of coliform organisms a positive Voges and Proskauer's reaction was given only by certain members of subgroups 3 and 4 of the *B. coli* group. All the other bacilli tested were negative (Table VIII).

Table VIII.

The Voges and Proskauer reaction in the B. coli *group.*

	No. of cases investigated	No. of cases V. and P. +	Percentage of cases V. and P. +
Subgroup 1	4	0	0
,, 2	3	0	0
,, 3	10	3	30
,, 4	13	10	77

In this series, therefore, it is found that all varieties which give the Voges and Proskauer reaction are saccharose fermenters, while of the 17 varieties which are V. and P. – 7 do not attack this sugar. A somewhat similar relationship exists between the Voges and Proskauer reaction and salicin fermentation. All V. and P. + organisms ferment salicin, but of the 17 V. and P. – varieties 11 only are salicin fermenters. No such correlation exists between the Voges and Proskauer reaction and dulcite fermentation. Of the 13 V. and P. + varieties, 3 or 23 % are dulcite +. These observations are in accordance with the findings of Levine (1916) and of Kligler (1914), both of whom found that salicin fermentation is more closely correlated to the Voges and Proskauer reaction than is dulcite fermentation. A much more interesting observation from the point of view of wound infection is made by Levine (1916). In studying 157 strains of *B. coli* (39 from sewage and 117 from faeces), he found that all those which gave the Voges and Proskauer reaction, nine in number, were derived from sewage, while none of the 117 organisms of faecal origin was positive to the Voges and Proskauer test. From this he concludes that the Voges reaction is characteristic of non-faecal strains of *B. coli*. A scrutiny of MacConkey's tables (1909) reveals figures confirming this view. Out of 497 lactose fermenting bacilli investigated, 91 were V. and P. +, and of these only 19, or 21 %, were of faecal origin. Of the 406 V. and P. – strains 332, or 81 %, were obtained from faeces. The chief sources from which the V. and P. + strains were obtained were soil, roof washings, pond water, rain water, grains, malt and cheese. The large number of V. and P. + organisms in the present series would therefore point to a non-faecal source for at least a considerable proportion of the coliform bacilli infecting these wounds, and the probable nature of such previous habitat is indicated by the authors quoted. A similar conclusion is arrived at by a study of the subgroup distribution of the members of the *B. coli* group. In Table IX the proportion of strains (not varieties) of lactose fermenters from wounds falling into each of MacConkey's subgroups is shown, and a comparison instituted with the organisms isolated by MacConkey from, on the one hand, human, cow and horse faeces, and on the other cesspool sewage, soil, pond water, rain water, and roof washings. From this it would appear that, even as regards lactose fermenters alone, wound coliforms occupy an intermediate position to the other two, but have on the whole a closer resemblance to the non-faecal than to the faecal series.

TABLE IX.

Subgroup distribution of strains of B. coli *from* (1) *wounds*, (2) *faeces*, (3) *non-faecal sources* (*per cent.*).

	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4
1. Lactose fermenters from wounds (M. J. S.)	14·6	12·2	36·6	36·6
2. Lactose fermenters from human, cow and horse faeces (MacConkey)	17·8	31·9	39·6	10·7
3. Lactose fermenters from sewage, soil and surface waters, etc. (MacConkey)	8·4	4·2	49·3	38·1

Notes on individual members of the B. coli *group* (Table XIII). (The numbers refer to the varieties described in Table XIII.)

2 and 4 (4 strains) give the reactions of *B. vesiculosus*. They differ only in that 2 ferments salicin while 4 does not.

3 (1 strain) gives the reactions of *B. grünthal* except that it is dextrin −.

5 (1 strain) resembles *B. vesiculosus* but does not form indol.

6 (2 strains) gives the reactions of *B. coli communis*.

7 (2 strains) is similar but non-motile (*B. coli immobilis*).

10 (2 strains) resembles *B. pneumoniae* (Friedländer) in every respect except that it gives the Voges and Proskauer reaction.

12 (1 strain) is interesting as being the only inulin fermenter in the whole series.

14 (4 strains) gives the reactions of *B. coli communior* (*B. pseudocoli* Castellani, 1912). One organism was tested on and found to ferment amygdalin, with formation of acid only.

15 (2 strains) = *B. neapolitanus*.

16 (1 strain) differs from *B. coli communior* only in that it does not ferment sorbite.

17 (1 strain) is the same as *B. MacConkey* No. 74, which was originally isolated from human faeces.

19 (2 strains) differs from *B. neapolitanus* only in that it does not ferment salicin.

22 (2 strains) = *B. MacConkey* No. 102. This organism was twice isolated by MacConkey from pond water.

23 (1 strain) = *B. lactis aerogenes* (Lewis, 1916). Indol is formed.

24 (3 strains) = *B. lactis aerogenes* (Castellani, MacConkey, etc.). Indol is not formed. One strain only was tested on amydalin and was found to form acid but not gas.

25 (2 strains) is the same as No. 22 (*B. MacConkey* 102) except that it does not ferment glycerin.

26 (1 strain) is the same as 25 except that it is non-motile.

27 and 29 (2 strains) give the same reactions as *B. cloacae*, but one ferments glycerin the other not. They are salicin +, but Castellani gives *B. cloacae* as salicin −.

31 (2 strains) = *B. coscoroba*, but is salicin +.

B. proteus Group.

Under this head 29 organisms, or 19·6 % of the total, are grouped, of which no fewer than 25 strains give identical reactions (No. 37 in Table XIII). These, which may be taken as the reactions of *B. proteus vulgaris*, are as follows: (1) fermentation (with production of gas in some, or more usually in all, of the tubes) of glucose, saccharose, laevulose, galactose and glycerin, (2) non-fermentation of all other substances tested, (3) rapid liquefaction of gelatin, (4) motility, usually very active, (5) non-production of indol, (6) absence of the Voges and Proskauer reaction, (7) on litmus milk variable reactions leading to clotting, bleaching, and finally digestion of the clot. The variations from this typical formula are very slight. Two strains are apparently non-motile (No. 38), and two ferment maltose (Nos. 35 and 36). No. 35 in addition forms indol, the only one out of 29 found to possess this property.

Members of the group were isolated from 29 cases, giving a case incidence of 24 %, almost equal to that of the *B. coli* group itself. As to the significance of these organisms in wounds, it may be asserted confidently that they are evidence of non-faecal contamination. The *Bacillus proteus* is commonly found in connection with putrefactive processes generally, and apart from this is apparently widely distributed in nature. It is, in my experience, distinctly uncommon as an inhabitant of the colon in man. In the course of a recent investigation of several thousand samples of faeces from dysentery convalescents I have isolated a *proteus* bacillus less than a dozen times. As a pathogenic agent this organism is now well recognised.

B. Morgan No. 1.

Seven organisms give the fermentation reactions of *B. Morgan* No. 1 (Morgan, 1906), and of these four are classical in every respect. These organisms ferment glucose, laevulose and galactose only, with formation of acid and gas, and they form abundant indol on peptone water. Gelatin is not liquefied, and they do not give the Voges and Proskauer reaction. The four classical examples in this series are motile, and render litmus milk strongly alkaline after a variable number of days. The remaining three are apparently non-motile, and they produce no change in litmus milk, even after three weeks. An apparently identical non-motile organism has been isolated by MacConkey (MacConkey, 1909) from horse faeces. *B. Morgan* No. 1 is of course a fairly common faecal organism in man, apart altogether from the diarrhoeal conditions in

which its occurrence was first observed by Morgan, and its presence in these wounds is presumably to be taken as evidence of excretal contamination.

B. faecalis alkaligenes.

This organism was isolated on one occasion only. It is motile, does not ferment any of the substances tested, and neither forms indol nor liquefies gelatin. The Voges and Proskauer reaction is not given. It produces intense late alkalinity on litmus milk. It also is presumably derived from a faecal source.

Group X.

This is a fairly compact group of eight organisms, separable into three varieties, giving rather peculiar reactions. Five strains are apparently identical (No. 43 in Table XIII), and may be described first. Glucose, saccharose, laevulose, galactose and inosite are fermented, with formation of acid only. None of the other 15 substances tested are attacked. Gelatin liquefaction is slowly produced, only commencing in from two to six weeks after inoculation. By the end of two months from ½ in. to 1 in. of a gelatin stab is liquefied, the whole tube only in from three to four months. The organism is actively motile and it forms abundant indol in peptone water. The Voges and Proskauer reaction is not obtained. On litmus milk the medium is first rendered slightly acid, and this lasts for several days. The reaction then slowly changes to alkaline, and this may be accompanied by a certain amount of bleaching. Alkalinity is usually well marked in from seven to fourteen days, but in one case it only developed during the third week, the medium having first been completely decolorised. Clotting does not occur at any stage.

It will be noticed that, while in its fermentation reactions and certain other features this organism closely resembles *B. proteus vulgaris*, it differs from it in certain important characteristics, viz.: (1) in the absence of gas production in its fermentation of the sugars, (2) in the fermentation of inosite, (3) in the absence of glycerin fermentation, (4) in the formation of abundant indol, (5) in its slow liquefaction of gelatin, and (6) in its alkaline reaction in litmus milk, with absence of clotting and digestion of the clot.

The three remaining strains in this group differ from those already described on one or two points only. The chief distinction is that gelatin is not liquefied. Two strains (No. 43) ferment glycerin, and one (No. 44) fails to ferment saccharose.

I have failed to find any reference to organisms giving these reactions, but several of Castellani's (Castellani, 1912) Ceylon strains (of faecal origin) differ on a few points only from the non-liquefying members of this group (*B. negombensis* and *B. talavensis*). The whole group, and especially the non-liquefying members, bear a certain resemblance to *B. Morgan* No. 1. The chief points of distinction are (1) the absence of gas in the fermented sugars, (2) the fermentation of inosite, (3) the liquefaction of gelatin by No. 42, (4) the fermentation of glycerin by No. 43, and (5) the fermentation of saccharose by both 42 and 43. In the case of No. 44, therefore, which is saccharose –, glycerin –, and gelatin –, the resemblance is a fairly close one.

It is an interesting fact that all three varieties were, in one instance (case 4), isolated from a single wound, Nos. 42 and 44 at the first examination, No. 43 at a second examination 70 days later. Probably this is an example of bacterial mutation (*vide infra*).

It may be remarked here that in the present series of organisms fermentation of inosite occurred in 6 out of 31 varieties tested, viz. the three varieties of Group X just described, two varieties of *B. lactis aerogenes*, and the very atypical organism No. 49. Formation of gas occurred only in the case of *B. lactis aerogenes*.

Group Y.

26 organisms, or 17·6 % of all those studied, were found to belong to a very well defined and compact group, the essential common characters of which are these: galactose A, laevulose –, motility –, indol –, and Voges and Proskauer –. The only variable characters are the action on gelatin and on glucose. None of the other carbohydrates, etc., tested are fermented. These organisms grow readily on bile salt, neutral red, crystal violet agar, forming in 24 hours medium sized colonies of a light pink colour with greenish fluorescence. On agar plates the individual colonies are also fluorescent, while stroke subcultures on agar slopes give rise to a thick white growth within 24 hours. All the members of the group show, when subcultured on the carbohydrate broths, a striking bluish or greenish iridescent ring on the side of the test tube, immediately above the upper level of the meniscus. According to their action on glucose and on gelatin the group has been divided into four varieties, thus:

					No. of strains
I.	Glucose	A	Gelatin	+	12
II.	,,	A	,,	–	3
III.	,,	–	,,	+	10
IV.	,,	–	,,	–	2

Growth on gelatin.

A. Non-liquefiers (varieties II and IV).

Gelatin stab cultures grown at 22° C. show, after a day or two, a filiform growth along the needle track. In the course of three or four weeks the stab may become slightly beaded, or villous outgrowths may take place from the upper part, but growth is never very prolific. On the surface a much more abundant growth takes place, forming a thick, greyish white layer.

B. Liquefiers (varieties I and III).

For the first two or three weeks the gelatin stab culture develops in a similar fashion to that described above, being either filiform or very finely beaded, with a thick growth on the surface. Liquefaction usually starts in the third or fourth week, occasionally slighter later, and forms at first a tiny cup beneath the central part of the thick surface layer. Liquefaction progresses very slowly downwards, but reaches the side of the tube in the course of a few days. In two months' time about ¼ in. is usually liquefied, and in four months about ½ in. When liquefaction occurs, the thick surface layer of growth remains *in situ* as a pellicle, while an abundant dense deposit falls to the bottom. The liquefied portion of gelatin is usually clear, but may contain flocculi. In a few cases liquefaction commences only after about six weeks, and progresses even more slowly than above described.

Growth on litmus milk.

All the members of the group gave closely similar reactions with litmus milk. The differences between individual strains were slight and unimportant, and referred chiefly to the rate of production of the various changes. The first noticeable change was either slight bleaching or slight acidification, or a combination of the two, and took place as a rule on the third or fourth day. In the case of one organism the milk became slightly bleached within the first 24 hours, in seven strains the first changes were noticed within 48 hours, while in three no change was observable until the fifth day. In 24 out of the 27 strains clotting occurred in from three to ten days. The three exceptions to this showed only complete bleaching at the end of seven days, but all clotted during the course of the second week. In some cases clotting was preceded or accompanied by slight reddening of the litmus, and usually by more or less bleaching as well. In others clotting was preceded or accompanied by bleaching only, usually complete. At the end of a fortnight all milks

were firmly clotted and litmus completely bleached, except that in the great majority the top $\frac{1}{8}$ in. to $\frac{1}{4}$ in. of clot had become strongly acid.

I have never come across a faecal coliform giving these reactions, and it seems probable that these organisms are derived from soil and water. In some respects they resemble Jordan's (1903) "*Fluorescens*" group, but they are quite non-motile and the gelatin liquefiers act only very slowly. Castellani (1912) has described an organism (*B. gintotensis*) having characters identical with variety II in the above table, except that it produces acid with arabinose in addition to its action on glucose and galactose. It was obtained from the faeces of a native of Ceylon.

Unclassified Coliforms.

No. 49 (Table XIII). This very extraordinary organism ferments glucose, saccharose, mannite, adonite, laevulose, galactose, isodulcite, inosite, and erythrite, with formation of acid but no gas. None of the other substances is attacked. It is the only organism in the whole of this series which ferments erythrite. Litmus milk is rendered alkaline after four or five days, and then clotted and bleached, with finally solution of the clot. It does not liquefy gelatin nor form indol: it is non-motile and the Voges and Proskauer reaction is not given. An organism having somewhat similar characters was obtained by MacConkey from a specimen of human sputum. It did not ferment saccharose, however, and it formed gas on adonite. It may be noted that organism No. 49 formed acid on saccharose only after 14 days. Another closely similar organism (*B. kandiensis*) was obtained by Castellani from the faeces of a healthy native of Ceylon. It differs from No. 49 only in being motile and in fermenting glycerin.

No. 50. This organism closely resembles the third variety of Group Y (No. 47), from which it differs only in that it is actively motile and is a rapid liquefier of gelatin.

Nos. 51 *and* 52. Like *B. faecalis alkaligenes*, these fail to ferment any of the sugars, but both liquefy gelatin, and while No. 51 is motile No. 52 is not. On litmus milk No. 51 produces early clotting and bleaching, No. 52 the same result after a preliminary stage of alkalinity. In other respects they are the same as *B. faecalis alkaligenes*. No. 51 corresponds to the *B. fluorescens liquifaciens* of Jordan (1903), and an apparently identical organism was isolated by MacConkey from faeces and tap water, and by Wilson (1908) from the urine.

General Observations.

I. *Group distribution.*

Table X shows the group distribution of the 148 strains of coliform bacilli studied, the number of varieties occurring in each group, and the case incidence in the 122 wounds examined.

TABLE X (showing group distribution).

Group	No. of strains	No. of varieties	Case incidence
1. *B. coli*	49	34	26 %
2. *B. proteus*	29	4	24
3. *B. Morgan* No. 1	7	2	5·7
4. *B. faecalis alkaligenes* ...	1	1	·8
5. Group X	8	3	5
6. Group Y	26	4	20
7. *B. pyocyaneus* ...	24	1	20
8. Unclassified	4	4	3

These figures may be compared with the results obtained by Dudgeon, Gardner and Bawtree (1913) in the investigation of 100 wounds.

True "Colon" bacillus	11 %
"Coliform" bacilli ...	15 %
True *B. proteus* ...	5 %
Atypical *proteus* ...	2 %
B. pyocyaneus ...	1 %

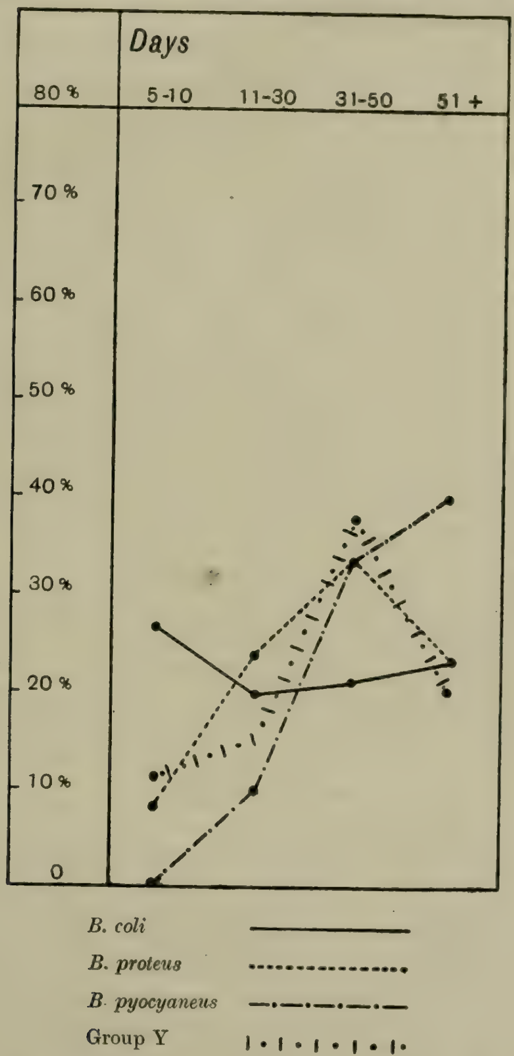
Goadby (1916), in the examination of 200 wounds, found *B. coli* present in 40 % and *B. proteus* in 47 % of cases, while *B. pyocyaneus* "was isolated on many occasions."

II. *Group distribution in relation to the age of the wound.*

It is of interest to observe the frequency of occurrence of these various bacterial groups in wounds of different ages, and for this purpose the wounds have been classified as follows: (A) 5 to 10 days old, (B) 11 to 30 days old, (C) 31 to 50 days old, and (D) over 51 days old. The result is shown in the accompanying Chart and in Table XI.

Members of the *B. coli* group have thus been recovered from a considerable proportion of wounds (20 % to 27 %) at all age periods. The curves of the *proteus* and Y groups show a remarkable parallelism. Starting at a low level (8 % and 11 %) during the first age period, they rise steadily to 34 % and 38 % respectively in the third age period, falling again considerably in the fourth. The *pyocyaneus* curve differs from both the foregoing. This organism, which did not occur once during the first age period, rose steadily thereafter to a maximum of 40 % in the fourth age period. It would be interesting to know the

CHART. *Incidence of various coliform bacilli at different age periods.*



incidence of infection by these organisms during the first five days. Distaso (1916) has expressed the opinion that infection by the *B. coli* group is probably constant in the earliest stages of wounds but has not been described, as "the soldiers come for observation too late and the phase is one which passes quickly." The author recognises a second phase in which "the *B. coliformis* (i.e. the *B. coli* group only) disappears,

TABLE XI.

Incidence of various coliform bacilli in wounds of different ages.

Age period	No. of wounds examined*	Percentage infected with coliform bac.	Percentage infected with these groups: 1 *Coli*	2 *Proteus*	3 Y	4 *Pyocyaneus*
5 to 10 days	37	40	27	8	11	0
11 to 30 days	39	48	20	23	15	10
31 to 50 days	29	83	21	34	38	34
Over 51 days	30	77	23	23	20	40

* In this table, 13 wounds which were examined on a second occasion after a considerable interval are each entered as two.

and the field is occupied by the anaerobic microbes of putrefaction," and a third phase in which cocci and the *B. subtilis* group only are present. He also expresses the opinion that the coliform organisms described in wounds in the late phase are either *B. proteus* or *B. pyocyaneus*. The observations recorded in the present communication show that some of these statements are only partially true. It is found, for example, that members of the *B. coli* group are present in a considerable proportion of wounds at all stages, but in the later phases *B. proteus* and *B. pyocyaneus* certainly occur with greater frequency. The continuous rise in the curve of *B. pyocyaneus* and the absence of this organism from wounds in the earliest stages, clearly indicate that this is of the nature of a "hospital infection."

Goadby (1916) has examined wounds from the earliest stages onwards, and his results as regards the *coli* and *proteus* groups do not by any means support the views advanced by Distaso (Table XII).

TABLE XII.

Incidence of coli *and* proteus *infection of wounds* (*Goadby*).

Age period	Total cases examined	Percentage infected: (1) *B. coli*	(2) *B. proteus*
1–8 days	64	39	50
5–10 days	57	33	42
11–30 days	40	47	47
Over 31 days	39	43	51

Contamination of wounds by fresh human faeces, on which Distaso lays great stress, is certainly indicated in a considerable number of cases by the presence of such definitely faecal organisms as *B. coli communis*, *B. neapolitanus*, *B. faecalis alkaligenes*, *B. Morgan* No. 1 and others. The great majority of the coliforms present, however, are probably derived from non-faecal sources, e.g. the *proteus* group, the Voges and Proskauer-positive members of the *coli* group, Group Y, and *B. pyocyaneus*.

It is worthy of note that no organisms giving the fermentation reactions of the Paratyphoid-Gaertner group have been obtained from any of the wounds.

III. *Observations bearing on the question of bacterial variation.*

In five cases out of 46 in which several varieties of coliform bacilli were isolated from one wound, it has happened that two of the strains present have differed from each other on one or two points only. In the light of recent work on bacterial variation (Twort, Penfold, etc.), it seems reasonable to suppose that we are here dealing with strains derived from a fairly recent common ancestor, and that variation has occurred by the acquisition of new characters or by the loss of old, or both. The instances referred to are these.

(1) In case 1 (Table III) two organisms of the *B. coli* group, subgroup 3 (Nos. 17 and 20 in Table XIII), were isolated. They differ from each other in respect of three characters only. No. 17 is salicin +, sorbite −, and indol −, while No. 20 is salicin −, sorbite +, and indol +.

(2) In case 3 two organisms of the *B. coli* group, subgroup 3 (Nos. 16 and 21 in Table XIII), were isolated, which vary only with regard to salicin fermentation, No. 16 being salicin +, No. 21 salicin −.

(3) In case 4 two organisms of Group X (Nos. 42 and 44) were isolated which differ on two points only. No. 42 forms acid on saccharose and liquefies gelatin, No. 44 does neither.

(4) In case 12 two closely similar organisms of the *B. coli* group (Nos. 6 and 14) were isolated. No. 6 is saccharose −, raffinose −, No. 14 saccharose +, raffinose +; otherwise they are identical.

(5) In case 64 two members of the *B. coli* group, subgroup 3 (Nos. 15 and 19), were isolated. They differ only in that No. 15 ferments salicin, while No. 19 does not.

In 11 cases from which coliform bacilli had already been recovered a second examination of the wound was made after an interval of some days or weeks, and in four of these the same organism was present on both occasions. In case 3 *B. Morgan* No. 1 (No. 39 in Table XIII) was thus obtained again after an interval of 47 days, in case 19 a member of Group Y (No. 47 in Table XIII) after 10 days, and in cases 31 and 37 *B. pyocyaneus* after intervals of 20 and 21 days. In two other instances (cases 6 and 21) a member of Group Y was isolated after intervals of 41 days and 44 days, the strains on each occasion differing only with regard to the fermentation of glucose. In case 4, as already mentioned, two closely similar organisms belonging to Group X (Nos. 42 and 44)

TABLE XIII.

The Biological Reactions of the Coliform Bacilli isolated from the Wounds of War.

Serial Number	Frequency of occurrence	Glucose	Lactose	Saccharose	Dulcite	Mannite	Adonite	Inulin	Salicin	Sorbite	Laevulose	Galactose	Raffinose	Maltose	Arabinose	Dextrin	Isodulcite	Inosite	Glycerin	Amygdalin	Erythrite	Litmus Milk Early		Litmus Milk Late	Gelatin	Motility	Indol	Voges and Proskauer's Reaction
B. COLI GROUP																												
Subgroup I (MacConkey)																												
1	1	+	+	−	−	+	−	−	+	+	+	+	.	+	.	+	.	.	+	.	.	A	.	AC	−	+	+	.
2 *B. vesiculosus*	3	+	+	−	−	+	−	−	+	+	+	+	−	+	+	+	+	−	+	−	−	AC	.	CB	−	−	.	−
3	1	+	+	−	−	+	−	−	+	+	+	+	.	+	+	−	.	−	+	.	.	AC	.	CB	−	+	.	−
4 *B. vesiculosus*	1	+	+	−	−	+	−	−	−	+	+	+	.	+	+	+	.	−	+	.	.	AC	.	ACB	−	−	.	−
5	1	+	+	−	−	+	−	−	−	+	+	+	−	+	+	+	+	.	+	−	−	AC	.	ACB	−	−	−	−
Subgroup II (MacConkey)																												
6 *B. coli communis*	2	+	+	−	+	+	−	−	+	+	+	+	−	+	+	+	+	−	+	−	−	AC	.	ACB	−	+	+	−
7 *B. coli immobilis*	2	+	+	−	+	+	−	−	+	+	+	+	.	.	+	+	+	−	+	.	−	AC	.	ACB	−	−	+	−
8	1	+	+	−	−	+	−	−	−	+	+	+	.	+	.	+	.	−	+	.	.	AC	.	ACB	−	+	−	.
9	1	+	+	−	+	+	−	−	−	+	+	+	.	+	+	−	+	.	+	.	−	AC	.	ACB	−	−	+	−
Subgroup III (MacConkey)																												
10	2	+	+	+	+	+	+	−	+	+	+	+	.	+	.	+	.	.	+	.	.	A	AC	CB	−	−	−	+*
11	1	+	+	−	+	+	+	−	−	+	+	+	.	+	.	+	.	.	+	.	.	AC	AC	CB	−	−	+	.
12	1	+	+	+	+	+	−	+	+	+	+	+	.	+	.	+	.	.	−	.	.	A	AC	CB	+	+	.	+
13	1	+	+	+	+	+	−	−	+	+	+	+	.	+	.	+	+	.	+	.	−	CB	.	ACB	+	−	.	+
14 *B. coli communior*†	4	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	+	A*	−	AC	.	CB	−	.	.	−
15 *B. neapolitanus*	2	+	+	+	+	+	−	−	+	+	+	+	.	+	+	+	+	−	+	.	−	AC	.	ACB	−	−	.	−
16	1	+	+	+	+	+	−	−	+	−	+	+	.	+	.	+	.	.	+	.	.	AC	.	ACB	−	+	.	−
17	1	+	+	+	+	+	−	−	+	−	+	+	.	+	.	−	.	.	+	.	.	AC	.	CB	−	+	−	−
18	1	+	+	+	+	+	−	−	−	+	+	+	.	+	.	+	+	.	+	.	−	AC	.	ACB	−	+	+	.
19	2	+	+	+	+	+	−	−	−	+	+	+	.	+	+	+	+	.	+	.	−	AC	.	ACB	−	−	+	−
20	1	+	+	+	+	+	−	−	−	+	+	+	.	+	+	−	.	−	+	.	.	AC	.	CB	−	.	+	−
21	1	+	+	+	+	+	−	−	−	−	+	+	.	+	.	+	.	.	+	.	.	AC	.	AC	−	+	+	−
Subgroup IV (MacConkey)																												
22	2	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	A	.	AC	+	+	−	+
23 *B. lactis aerogenes*‡	1	+	+	+	−	+	+	−	+	+	+	+	.	+	.	+	.	+	+	.	.	A	.	ACB	−	−	+	+
24 *B. lactis aerogenes*§	3	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	A*	−	AC	.	CB	−	−	−	+
25	2	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	A	.	AC	+	+	−	+
26	1	+	+	+	−	+	+	−	+	+	+	+	.	+	.	+	+	.	−	.	−	A	.	CB	+	−	−	+

27	*B. cloacae*	1	+	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+	−	+	A	−	A	.	AC	+	+	−	+
28		1	+	+	+	−	+	−	−	+	+	+	+	.	+	+	+	+	.	+	.	−	AC	.	CB	−	−	−	+
29	*B. cloacae*	1	+	+	+	−	+	−	−	+	+	+	+	.	+	+	+	+	.	−	.	−	A	AC	CB	+	+	−	+
30		1	+	+	+	−	+	−	−	+	+	+	+	−	+	+	+	−	.	+	.	−	A	.	AC	+	+	+	−
31		2	+	+	+	−	+	−	−	+	+	+	+	.	+	.	+	−	.	+	.	−	Alk	A	AC	−	−	+	−
32		1	+	+	+	−	+	−	−	+	+	+	+	.	+	+	+	.	−	−	.	−	A	.	ACB	−	+	−	+
33		1	+	+	+	−	+	−	−	+	−	+	+	+	+	+	+	+	.	−	A	−	A	.	AC	+	+	−	+
34		1	A	A	A	−	A	−	−	A	−	A	A	−	A	A	A	−	−	A	−	−	A	.	AC	+	+	−	−
B. PROTEUS GROUP																													
35		1	+	−	+	−	−	−	−	−	−	+	+	.	+	.	−	.	.	+	.	.	A	AC	CBD	+	+	+	−
36		1	+	−	+	−	−	−	−	−	−	+	+	.	+	−	−	.	−	+	.	.	Alk	.	CBD	+	+	−	−
37	*B. proteus vulgaris*	25	+	−	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	A Alk	.	CBD	+	+	−	−
38		2	+	−	+	−	−	−	−	−	−	+	+	.	−	.	−	.	−	+	.	.	A	AC	CBD	+	−	−	−
B. MORGAN No. 1																													
39		4	+	−	−	−	−	−	−	−	−	+	+	.	−	−	−	.	−	−	.	.	− Alk	.	Alk	−	+	+	−
40		3	+	−	−	−	−	−	−	−	−	+	+	.	−	−	−	.	−	−	.	.	−	.	−	−	−	+	−
B. FAECALIS ALKALIGENES																													
41		1	−	−	−	−	−	−	−	−	−	−	−	.	−	−	−	.	−	−	.	.	−	.	Alk	−	+	−	−
GROUP X																													
42		5	A	−	A	−	−	−	−	−	−	A	A	−	−	−	−	−	A	−	−	−	A	B	Alk	+	+	+	−
43		2	A	−	A	−	−	−	−	−	−	A	A	.	−	.	−	.	A	A	.	.	A	.	Alk	−	+	+	.
44		1	A	−	−	−	−	−	−	−	−	A	A	−	−	−	−	.	A	−	.	.	A	B	Alk	−	+	+	−
GROUP Y																													
45		12	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	+	−	−	−
46		3	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	−	−	−	−
47		10	−	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	+	−	−	−
48		2	−	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	−	−	−	−
UNCLASSIFIED																													
49		1	A	−	A	−	A	A	−	−	−	A	A	−	−	−	−	A	A	−	−	A	Alk	.	BD	−	−	−	−
50		1	−	−	−	−	−	−	−	−	−	−	A	.	−	.	−	.	.	−	.	.	AC	.	CBD	+	+	−	.
51		1	−	−	−	−	−	−	−	−	−	−	−	.	−	.	−	.	.	−	.	.	CB	.	CB	+	+	−	.
52		1	−	−	−	−	−	−	−	−	−	−	−	.	−	.	−	−	.	−	.	.	Alk	B	CB	+	−	−	.

* One organism only tested. † *B. pseudocoli* (Castellani). ‡ Lewis. § Castellani.

A = Formation of acid. + = Formation of acid and gas, Liquefaction of gelatin, motility, Formation of indol, or a Voges and Proskauer's reaction.

AC = Acid and clot in litmus milk. B = Bleaching. CB = Clot and bleaching in litmus milk.

ACB = Acid, clot and bleaching in litmus milk. BD = Bleaching and digestion of clot. Alk = Alkalinity.

CBD = Clot, bleaching in litmus milk, digestion of clot.

were isolated on the first occasion, while on the second, after an interval of 70 days, an organism of the same group (No. 43) but differing slightly from both the others was obtained. Apart from these instances the organisms isolated on each second occasion appeared to bear no relationship whatever to those obtained at first. For example, in case 37 the first examination showed the presence of *B. Morgan* No. 1, the extremely atypical organism No. 49, *B. pyocyaneus*, and three varieties of *B. coli*. After an interval of 21 days there were present *B. pyocyaneus* and a member of Group Y.

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DENGUE FEVER IN AUSTRALIA.

ITS HISTORY AND CLINICAL COURSE, ITS EXPERIMENTAL TRANSMISSION BY *STEGOMYIA FASCIATA*, AND THE RESULTS OF INOCULATION AND OTHER EXPERIMENTS.

BY J. BURTON CLELAND, M.D., CH.M. (Sydney),
Principal Microbiologist, Department of Public Health, Sydney, New South Wales;

AND BURTON BRADLEY, M.B., CH.M. (Sydney), D.P.H. (London),
Sydney.

ASSISTED IN THE INOCULATION EXPERIMENTS BY

W. McDONALD, M.B., CH.M.,
Rookwood State Hospital and Asylum, Sydney.
(*From the Microbiological Laboratory, Department of Public Health, N.S.W.*)

(With IX Charts.)

CONTENTS.

INTRODUCTION.

Epidemic Dengue first reached Australia early in 1885. In the same year, according to Castellani and Chalmers in their *Manual of Tropical Medicine*, it reached the Fiji Islands, "to which it was conveyed by a European suffering from the complaint." As later on in the same year a record occurs of cases of dengue fever on a steamer which arrived in Sydney from Fiji and Noumea, it is possible that the disease reached Australia from Fiji. Since this date, from time to time very extensive epidemics of dengue have occurred in Queensland, sometimes extending to the northern coastal towns of New South Wales.

A careful comparison of previous *clinical* descriptions of the epidemic disease known as dengue in Australia, with the description of the disease compiled from various sources as given in Castellani and Chalmers (*loc. cit.*), does not reveal anything tangible to suggest that more than one disease has, up to the present, been comprised under the term "dengue fever." The only important clinical difference appears to be that in the Australian disease, though the pulse varies more or less with the temperature, it is nevertheless relatively slow, and sometimes absolutely so[1].

Elsewhere in this report, chiefly as a result of our investigations, will be discussed the question as to whether or not under the broad term "dengue" it is possible that more that one distinct entity has hitherto been included.

Origin of these experiments.

In March, 1916, an extensive epidemic of dengue, then prevalent in Queensland, reached some of the north coast towns of New South Wales. The incidence on the population was exceedingly heavy, and business was greatly disorganised in consequence. As the experiments into its means of spread in Australia hitherto carried out had been few and inconclusive, it was considered advisable to visit the area affected and collect material there for further study of the disease. It was recognised that if infective material could be conveyed to Sydney, a town in which indigenous cases of the disease have never been known to arise, results might be obtained which would be free from fallacies attendant on experiments conducted in the epidemic area. At the beginning of April one of us, therefore, with an assistant, paid a visit to Mirwillumbah.

[1] A discussion of the attempted differentiation between Dengue and Pseudo-Dengue with special reference to the views of Rogers and Grall will be found in the recent monograph of G. Sticker, *Dengue und andere endemische Küstenfieber*, pp. 76, Wien, 1914. This work supplies an extensive bibliography of the literature, both clinical, epidemiological and experimental. (Editorial note.)

He found that though the chief incidence of the disease had passed, there were still numerous cases, and that two species of mosquitoes were very abundant in the town, namely, *Culex fatigans* and *Stegomyia fasciata*. He collected a number of both species of these mosquitoes, more especially from houses in which cases of dengue had occurred and preferably in the actual rooms of patients then ill with the disease. In addition he withdrew specimens of blood from patients suffering from the disease, some of them being allowed to clot and some being received in citrated normal saline solution. The materials thus obtained were brought back to Sydney.

At this early stage of the investigations, the following main objects were held in view:

1. To try to transmit the disease to human volunteers by means of one or other of the two species of mosquitoes which had been captured in the epidemic area.

2. To attempt to establish by inoculation of material from the blood a strain of the disease for further study as to the incubation period, symptoms and signs, immunity, etc.

With the mosquitoes in the first instance conveyed to Sydney, amongst which there had been a heavy mortality, successful transmission of the disease was not achieved. However, by the inoculation of materials obtained from the cases in the epidemic area and from the blood of a patient who had contracted the disease in the epidemic area and had reached Sydney before he had recovered, and from the blood of one of us who had made the journey and who had contracted the disease in the epidemic area and had developed it after his return to Sydney, strains of the disease were successfully established in Sydney. Though unfortunately in the early cases some of the volunteers received inoculations of blood from two different sources, thereby obscuring certain data in connection with their cases, the main point attempted at this stage was achieved, namely, the establishment in human beings of strains of the disease by inoculation. The bulk of the experiments detailed later consisted of sub inoculations from these primary inoculation cases. It has been considered advisable to tabulate in full, in the shape of an appendix, each individual in chronological sequence on whom experiments were made. Under various headings, the results of these experiments are discussed as a whole with the object of ascertaining what information of value in connection with the disease and indirectly with other similar diseases can be reasonably deduced from the results obtained.

The failure to transmit the disease by the first batch of mosquitoes brought down from the epidemic area led to another of us with an assistant visiting in May the adjacent town of Mullumbimby then suffering heavily from the epidemic. A further considerable number of *Culex fatigans* and *Stegomyia fasciata* were brought back to Sydney and the typical disease was conveyed by the bites of the batch of *Stegomyia fasciata* to four volunteers in Sydney, thus establishing conclusively the rôle that this mosquito can play in the spread of the disease.

I. THE HISTORY OF DENGUE FEVER IN AUSTRALIA WITH A SHORT SUMMARY OF THE CLINICAL DESCRIPTIONS OF PREVIOUS EPIDEMICS[1].

The first reference to the occurrence of cases of this disease in Australia appeared in the *Aust. Med. Journ.* of 1873 (XVIII. 160) in the statement that eight cases of dengue, contracted at Mauritius, had occurred on board the ship "Charles Auguste" during the last few weeks of May. (Melbourne.)

In 1885 a note is found in the *Australasian Medical Gazette* (April, 1885, p. 177) to the effect that the disease was prevalent in Rockhampton (Queensland) to an alarming extent. In the same year (*Aust. Med. Gaz.* September, 1885, p. 310) five cases of dengue fever are reported as being discovered on the A.S.N. Co.'s steamer "Gunga" which arrived in Sydney from Fiji and Noumea on August 17th.

In 1886 Dr J. A. Weber, of Natimuk, Victoria (*Aust. Med. Journ.* July, VIII. 291) reported an outbreak which was thought to be typhoid fever when the cases were first taken to the hospital, but which, after consultation with the Board of Health, was definitely considered to be dengue. He had seen about thirty-two cases during the previous four months. The incubation period was not discovered. There was no prodromal stage. Quite suddenly the patient was attacked with severe pains all over the body as if the bones were broken ("Break-bone fever"); there were severe headaches, and a most characteristic pain in the eyeballs, increased greatly on slight pressure. The temperature was 103° F., seldom higher, 104° being the highest recorded. The face was red and swollen, and there was also some slight swelling of the joints, and

[1] This section was contributed by one of us (J. B. C.) to the *Third Report of the Govt. Bureau of Microbiology* dealing with the work performed during the year 1912. Sydney, New South Wales, Govt. Printer, 1914. As this publication is inaccessible to many readers and the subject-matter is so applicable to the present Report, it is reproduced here in full.

frequently an erythematous eruption all over the body. There were also constipation and loss of appetite.

The symptoms gradually subsided in about three days. Relapses were frequent, usually about the seventh day, but were unaccompanied by redness or swelling over the face or body. Without complication, patients recovered at the end of eight days. Redness and swelling of the face were constant in all the cases, whilst erythema and swelling of the joints were only transitory. There were three cases of relapses, and one in which premature labour developed.

In 1894 the disease was prevalent in Thursday Island, nearly every male and the majority of the females suffering from it (*Aust. Med. Gaz.* July, 1894, p. 252). In 1895 it was very prevalent in Townsville (*Aust. Med. Gaz.* February, 1895, p. 79). In 1897 an extensive epidemic occurred in Queensland, which produced a discussion before the Medical Society of Queensland on 4th May (*Aust. Med. Gaz.* May, 1897, p. 235). In this discussion, Dr Thomson described the complaint as follows:

"It consisted of a febricula combined with an erythema, many cases being of very mild character. In some, however, the temperature reached 103° or 104° F. but this was always followed by a rapid defervescence. Pains were sometimes complained of in the muscles and joints. In nearly every case there was a cutaneous eruption, sometimes of a measly character; sometimes an erythema; and usually of a patchy nature. In some cases it was only visible on the thighs. Desquamation did not occur, and no serious results followed. The patients were left very weak, and convalescence was prolonged out of all proportion to the severity of the attack. In a few cases catarrhal symptoms, resembling those of influenza, were present. The disease was certainly infectious."

Dr Hopkins was of opinion that the disease was very like influenza, and that it did not correspond to the symptoms of dengue as given in the text books.

Other speakers referred to the rash being commoner on the forearms—in one case only on the palms of the hands—and sometimes of a type intermediate in character between that of scarlet fever and that of measles. One observer had occasionally seen some desquamation.

Dr Halford said that the Brisbane Hospital had been inundated with cases; that the peculiarity of the rash was its multiformity, being sometimes macular, papular, diffuse, or punctiform; that some cases had a very slow pulse (36 to 40); that some had a rash without any malaise; and that half the cases shewed some increase of splenic dulness.

Dr Turner was of the opinion that only a very few cases had symptoms resembling somewhat those of dengue fever, but that the great majority of them shewed no resemblance beyond the presence of a rash. He said that the term "breakbone fever" could not be applied to the cases he had seen, and that it was impossible to identify the epidemic as that described in books as dengue, and these descriptions were misleading.

Dr C. S. Hawkes, of Rockhampton (Queensland), in a paper in the *Inter. Med. Journ.* (1897, II. 450) describes cases met with in the epidemic of Central Queensland in the early part of 1897. In one case—that of a patient who lived in a neighbouring township and on one occasion visited Rockhampton and returned, no other cases arising—the incubation period was definitely ascertained to be three days. The disease itself was very variable. The prodromal symptoms were sometimes absent; sometimes consisted of malaise and rise of temperature to 99° or 100° F. over a period of from twenty-four hours to fourteen days. The onset was usually sudden, with severe headache, often shivering, but rarely a well-marked rigor; then followed aching of the limbs, backache, prostration in severe cases, and the initial rash or a deep flushing of the face, with a hot and dry skin. The course of an ordinary attack is given as follows: "The pains and fever increase during the first twenty-four hours, aching being felt in all the limbs, more in the neighbourhood of or in the joints, though patients find it very hard to localise their pains; backache is always present across the lumbar region, headache all over the head, often some pain in the eyes, which feel sore when moved; pain is also felt across the upper part of the abdomen, vomiting increases it somewhat, but it is often severe when none is present; vomiting more often comes on about the third or fourth day, but may in some cases be present from the first; the tongue coats with a whitish fur, the breath becomes offensive, and the initial rash, if present, may be noticed.

During the second day there is usually some remission of symptoms, if not of temperature, though there is occasionally a drop of two or three degrees about this time; the patient feels more comfortable, even if the temperature has not fallen; this remission does not last long, the temperature, if it has fallen, soon rises again, the pains return with greater intensity than before, retching and vomiting are present, with great dislike for food, often with restlessness and insomnia, these symptoms persisting with little variation till the crisis takes place at the end of the fifth day, or a little later.

In severe cases the crisis may be well marked, with vomiting,

diarrhoea, profuse sweating, bleeding from the nose and, more rarely, from the uterus, and occasionally delirium or, rarely, excessive drowsiness. As a rule, during the last day, the terminal rash appears on the body and limbs."

Hawkes describes fully abortive attacks; ordinary attacks ending in about five days and with temperatures ranging in severe cases to 104°, 106°, and even occasionally to 107° F.; cases lasting for seven or eight days and ending by lysis and usually less severe than the ordinary cases; and a type in which the fever is prolonged over several weeks or recurs at irregular intervals. The fall of temperature on the second day was not noticeable in this particular outbreak. Of other points noted may be mentioned a slow pulse-rate, in some cases at the beginning of the attack and during convalescence; the muscular nature of the pains (*e.g.* those of the eyeball); occasional severe and persistent vomiting; the rarity of troublesome salivation; "black vomit" in one case; the frequency of nose-bleeding at or near the crisis; insomnia and marked mental hebetude, and sometimes mental aberration, and even delusions of sight by day in the absence of delirium. Enlargement of the glands was uncommon, but in a few instances the posterior cervical glands and those over the mastoid felt a ittle increased in size. As sequelae, persistent pains in the limbs and the upper part of the abdomen and aching in the orbits were not uncommon. Some cases shewed dyspepsia, with dislike for food and disorders of taste, such as all food seeming bitter. In one case this bitterness was associated with a disagreeable foetid odour. There were also several cases of pleurisy, a few of catarrhal jaundice, some with periostitis, and a number with boils. Slight mental hebetude was common, and in a few cases mixed aphasia with misplacing of words.

The initial rash during the first day of fever appeared as a deep flush on the face and irregular dark-coloured blotches on the chest, or as an erythematoid mottling of the chest, neck, and face. It disappeared in the course of a few hours.

The terminal rash was more marked, and was usually scarletiniform or morbilliform, appearing first on the upper part of the chest, and accompanied with a sub cuticular mottling. A third type of rash was urticarial, sometimes appearing as a giant urticaria. With all forms there was some general irritation of the skin, itching of the hands and feet, and slight desquamation. Petechiae, sometimes extensive, occurred in some cases.

In 1898, Dr Eugen Hirschfeld, Honorary Physician to the Brisbane Hospital (*Intercol. Med. Journ.* 1898, III. 143) discusses in considerable

detail the two epidemics of dengue which had swept over Queensland from the north to the south during the previous twelve months. His paper should be consulted in the original.

This same epidemic of 1897 is discussed fully by Dr F. E. Hare, late of Charters Towers (*Aust. Med. Gaz.* March, 1898, p. 98), who collected valuable information from many sources. He points out that the epidemic commenced at Cooktown on 8th January, and reached Cairns on 8th February, and various other towns in North Queensland during March, Bowen not being attacked until 30th May; Herberton escaped. The whole paper is too long to quote here, but is well worthy of careful perusal. The information obtained is the result of forwarding circulars to most of the medical men in North Queensland, a number of whom replied.

A discussion on this paper appears in the same number of the *Aust. Med. Gazette,* p. 124, and a similar discussion which took place before the Medical Society of Queensland appears on page 130.

I find that this epidemic reached the northern districts of New South Wales in 1898 (*Ibid.* March, 1898, p. 135).

Dr J. Lockhardt Gibson, of Brisbane (*Ibid.* August, 1898, p. 339), describes a case of acute inflammatory glaucoma produced in a susceptible patient by dengue. The symptoms began on the second day of an acute attack of dengue.

In November, 1899, one death from dengue occurred in Brisbane (*Ibid.* January, 1900, p. 42), and two deaths are recorded in Brisbane in October, 1901 (*Ibid.* January, 1902, p. 46).

In the summer of 1904–5, a severe epidemic of dengue occurred in Queensland, on which a most complete report was compiled by a Committee appointed by the Queensland Branch of the British Medical Association. The members of this Committee were the President of the Branch (Dr Robertson) and Drs Thompson, Love, Turner and Wield. Their report appears in the *Aust. Med. Gaz.* (November, 1905, p. 616). As this report has been reprinted in the *Journ. Trop. Med.* 1905, VIII. 355, it will be unnecessary to do more than refer to it in this place.

Ninety-four cases were reported to have died from dengue in Brisbane out of a population of 125,672. The chief mortality occurred in March and April. If it could be assumed that half the population were attacked the mortality will stand as 1 in 668, if 75 per cent. were attacked 1 in 1003. An account is given of thirty-five fatal cases about which information was obtained. Some interesting information is appended

as to the spread of the disease from house to house in particular neighbourhoods.

This epidemic of 1904–5 reached Thursday Island in the latter part of January and the beginning of February (*Aust. Med. Gaz.* February, 1905, p. 91). Almost everyone was affected, but there was only one death—that of an infant. Several cases also occurred in the northern parts of New South Wales, as well as in Sydney (*Ibid.* April, 1905, p. 185). Dr Ashburton Thompson, in referring to this, said that the disease had many times been introduced into New South Wales in past years to his knowledge—the first time in 1886. It occurred when dengue was extremely prevalent in New Caledonia. It took place in August and September, when the weather was cool, but did not spread.

Dr T. L. Bancroft (*Ibid.* January, 1906, p. 17), in discussing the etiology of dengue, states that direct contact, *e.g.*, sleeping with a patient, will not give dengue. The intervention of an intermediate host is evident. As some people from the country visited friends in Brisbane by day and had got dengue, evidently *Culex fatigans* was not the agent, and probably *Stegomyia fasciata*—a day biter—was the culprit. Dr Bancroft experimented with the latter mosquito. Dengue occurred in one case after five days' incubation, the mosquito having been kept twelve days after biting. In another instance, a mosquito being kept ten days after biting, five days later a mild attack occurred. In two cases with a fifteen days' interval, and in one with a seventeen days' interval, nothing resulted.

Dr Fredk. Woolrabe, in a letter to the *Aust. Med. Gaz.* (February, 1906, p. 105), criticises Dr Bancroft's experiments, suggesting the possibility of natural infection in his positive cases.

Dr J. Lockhart Gibson (*Ibid.* May, 1906, p. 227, and Australasian Medical Congress, 1905, seventh session, p. 283), describes a case of keratitis in dengue. Three cases of keratitis dengue have come under his notice, and five cases of keratitis post-dengue. He says the dengue cases appeared to start as keratitis neuro-paralytica, but to this is added rapid infective ulceration of the affected cornea.

Dr R. A. O'Brien (*Ibid.* March, 1908, p. 121) states that clinical observation distinctly incriminates the *Stegomyia* (*Scutomyia*) *notoscripta*, the distribution of which was coincident with that of the dengue wave of three years ago. He says further that, since the wave of typical dengue, cases have been common in the north of Queensland resembling dengue in every detail, except for the absence of rash and the third-day remission. Recently typical cases had again begun to appear in those who had had

dengue three years ago, suggesting that the immunity was wearing out. These cases corresponded in blood picture to that described by Stitt and Balfour as existent in dengue, and in dengue only, viz., a leucopenia with a great initial drop in polymorphonuclears, as low as 35 per cent. in some cases that Dr O'Brien had, with a replacement by small lymphocytes, these in their turn giving place about the fourth or fifth day to large lymphocytes or large mononuclears.

As regards the occurrence of dengue in Western Australia, I have been informed by Dr J. H. Saunders that he has seen cases at Broome and Roeburne.

II. Clinical description of the 1916 epidemic of Dengue Fever on the North Coast of New South Wales.

The facts utilised in compiling this description were mostly obtained by observations and notes on cases seen by us on visits to the infected district, and by some observations on imported, mostly military, cases in Sydney. Thus our description is one largely of the symptomatology of the illness compiled from histories given us by patients, who, at the time, were suffering or had recently suffered from the disease. In particular we have little exact information as to the type of temperature or the pulse charts in the naturally occurring cases, and our descriptions of these are for the most part based on observations on our experimental mosquito-borne cases, or on what we have been told was the case by observers in the infected district. We have availed ourselves also of the excellent description by Goldsmid and Crosse[1] to which we refer the reader.

Onset. This, in the large majority of cases, is described as sudden. Frequently the patient will give the exact hour at which he was taken ill, and may narrate how before a certain time he was quite well, and that after an extremely short period, perhaps half-an-hour from the first symptom, he was prostrate with the disease. Out of thirty cases replying to questions as to the nature of the onset, twenty-five replied that it was sudden, three that it was gradual. In two cases the replies were doubtful.

The onset is usually accompanied by fever, headache, malaise and slight shivering, and to a greater or less extent by pains and aches, which are very characteristic in the typical cases. In certain cases the relation-

[1] Goldsmid and Crosse, Some notes on Dengue, *Med. Journ. of Australia*, May 6th, 1916, p. 377.

ship between the fever and other symptoms is less definite and the fever may precede or post-date the other symptoms.

Course. After the onset the disease runs a course lasting from a few days to a fortnight or more (four to seven days—Goldsmid and Crosse). There may be two periods of intensity of fever and symptoms, separated by a period, varying in length but usually only of a day or so, of comparative abeyance of fever and symptoms, during which the patient may regard himself as well. This double phase is, however, in our experience, by no means a constant phenomenon, and its absence cannot be regarded as militating against the diagnosis of dengue. Moreover, especially in mild cases, although a four-hour temperature chart may shew a distinct double phase variation, the symptoms and temperature do not always vary *pari passu*. In some cases there appears to be a tendency to relapse at a later period, but we have no very precise information on this point. In a typical case, after a sudden onset accompanied by a rapid rise of temperature, shivering and headache, and occasionally slight vomiting, the patient takes to bed with pains in the back and limbs and severe headache. He passes a very restless night and may be delirious. He finds it almost impossible to rest in any position. For the following day or so the headache and body pains are worse. The temperature soon falls, and this may be accompanied by sweating, and the patient gets up, not feeling very well, and with a dirty tongue and a residue of pains. One, two, or three days later the temperature goes up again and the symptoms return. The second attack lasts for one or two days, and then convalescence ensues. In the stage of onset there is usually an erythematous blushing of the skin, and later on, from the second to the seventh day, a more distinct rash frequently appears.

It will be best now to review *seriatim* the outstanding symptoms and signs of the disease as met with in the North Coast.

The temperature and pulse. We do not wish to discuss these fully at the present time as our investigations have not enabled us to take first-hand records of many natural cases. From the information we can gather, however, the double phase temperature is not constant, but inasmuch as few of the cases are in hospital where accurate records can be obtained we cannot dogmatise on this point.

Goldsmid and Crosse say "The temperature rose sharply and reached 101–103° F. During the course of the illness it remained high and did not undergo marked fluctuation. Not infrequently it reached 105° F. just before the termination of the fever. The fall was as rapid as the rise."

The pulse rate in natural cases has not come under our personal observation to any extent, but Goldsmid and Crosse confirm the results we obtained in our injection experiments. They say, "It (the pulse) was invariably slow in proportion to the temperature. A pulse rate of 75 to 90 was frequently associated with a temperature of 102° or 103° F. A more rapid pulse rate than 90 was rarely noted save just before the final fall of temperature."

The pulse rate and its relation to the temperature in experimental cases is discussed fully in a separate section.

The facies. The face soon assumes a very characteristic appearance, and in our experience this is one of the most useful signs of the disease. It looks red, swollen, hot and puffy. The eyes are usually somewhat injected, but there is not excessive lachrymation or any running at the nose. *Coryzal signs are notably absent* although it must not be forgotten that an ordinary "cold in the head" may coincide with an attack of dengue. Out of twenty-six cases questioned as to the occurrence of "running at the nose" only one described it as being present.

The facies of dengue has been described as resembling that of a person recovering from an alcoholic bout. It is also somewhat suggestive of the face in the early stage of measles but without the coryzal condition. The typical facies is most marked shortly after the onset, or, when this occurs, in the recrudescent period.

Headache. Headache is a practically constant phenomenon. Thus, out of twenty-six cases questioned all gave a history of headache. In some cases it was located as frontal; in others, as vertical or occipital; and quite frequently as "all over the head." Frontal headache is hard to distinguish from the characteristic eye pains, and the sufferer frequently refers to pains "at the back of the eyes." The intensity of the headache varies very much. In certain cases it appeared to be the principal cause of complaint, sometimes being described as "agonising," whilst in others it was referred to as slight.

Eye pains. The painful eyes are, in our opinion, quite one of the most characteristic single signs of dengue, and are almost always present in some degree. Sometimes the eyes are said to be aching severely and painful on movement, and we have seen cases where the whole head was turned rather than move the extremely sensitive eyes. In others, it is only by careful questioning that the presence of some pain or tenderness in the eye-balls or eye muscles is elicited.

Out of twenty-eight cases questioned, twenty-five described pains in the eyes and three denied their existence. Out of thirteen cases

questioned on the point, eleven said the eyes were painful to move, and two replied negatively.

Apparently the earlier symptom is pain in the eyes or in the "back of the eyes," easily confused with frontal headache. Later there is definite pain and tenderness apparently in the eyeballs, which is associated with pain on ocular movement and probably often with some photophobia.

Occasionally the eyes are described as "sore" which word may be used to refer to the irritation of slight conjunctival congestion, but conjunctival symptoms are never prominent and the adjective "sore" is probably frequently used to refer to the deeper-seated pains in the eyeballs.

General or body pains. These vary very much in degree, and are by some described as intense and agonising, and they may require the administration of morphine; in other cases they are described as "tired feelings," "gone in the knees," and "influenzal pains." In our experience the "break-bone" type of case is the exception, and the pains are, as a rule, not a very prominent feature. Sometimes their occurrence is denied in a particular case, or only elicited after careful questioning. Restlessness and inability to stop in one position is characteristic of some cases, and is probably closely linked to the body-pain symptoms. Out of thirty-two persons questioned, all described various degrees of *body pains* somewhere *in the spinal axis*, and out of twenty-six questioned, all described pains in the limbs. The back of the neck is a common seat of fairly severe pain (sixteen out of eighteen questioned). *Lumbo-sacral pain* is also common ("across the back" fourteen out of fourteen questioned). Real *pains in the joints* appear to be uncommon apart from the general limb ache. Movement does not seem definitely to increase the pains, but when severe the patient generally takes to bed because of the pain and associated symptoms. The body pains of dengue are in our opinion not associated with any readily demonstrable lesion. No swelling, redness or tenderness were noted in any case. This is in striking contrast with the description by Osler, who refers to red, swollen and painful joints.

Abdominal pains. These were described by ten out of a series of sixteen cases questioned and are sometimes associated with diarrhoea. At other times they are apparently a "spreading round" from the lumbar and dorsal region of the back pain. Pain in the epigastric region associated with vomiting is spoken of by Goldsmid and Crosse as occurring in several cases.

The body pains gradually subside, but there is usually a recrudescence of the pains when the second phase occurs. After the febrile stage is over there is generally some tiredness or aching for several weeks in the spine or limbs, which, however, gradually passes off.

Other nervous symptoms occurring in the disease are giddiness, delirium, mental irritability, depression and sleeplessness.

Giddiness is a common feature at various times in the course of the disease (nineteen out of twenty-two questioned).

Delirium is not frequent, but we have seen cases where there was maniacal delirium for three nights after the onset, and many cases shew some mental wandering when the temperature is high. Delirium was mentioned as a symptom in six out of twenty-one cases questioned on the point.

Mental irritability is a striking feature of the disease, especially in the later stages.

Depression. The depression following the attack is one of the most marked features, and the patient may be actually incapable of concentration or serious mental effort for a week or so after the attack.

Sleeplessness is found at some time in nearly all cases.

Gastro-intestinal symptoms are not marked. There is, however, a *dirty tongue* which is rather characteristic, being furred at the back with a strawberry tip very like that seen in scarlet fever. The *fauces* are reddened—Goldsmid and Crosse note a fine stippling of the soft palate as an early characteristic sign—and there may be some sore throat. This is usually not marked. *Anorexia* is a feature in most cases (thirty out of thirty questioned). *Nausea* is fairly common (eighteen out of twenty-nine questioned), and *vomiting*, though not as a rule marked, is met with especially at the onset (thirteen out of twenty-nine questioned) and occasionally may be severe. Some cases suffer no disturbance of the bowels, but *diarrhoea* is present in a few cases (two out of twenty-nine). *Constipation* is not general (four out of eleven).

Shivering occurs commonly (twenty out of twenty-four cases questioned). It may occur with the onset and during the febrile stage, but rigors are the exception.

The skin eruptions. According to Goldsmid and Crosse the preliminary and terminal rashes were well marked in cases seen by them. They note, however, that the preliminary rash could easily be overlooked. They describe this as a "fine punctiform rash usually found over points of friction....It appeared and disappeared very suddenly.... A fine stippling of the soft palate was often the only rash present when

the patients were first seen." They describe the terminal rash as "polymorphous," and as being present in nearly every case. "It was either papular or a dark red, blotchy rash, or an urticularia."

Our own experience probably covers a somewhat different type of case from that seen by Goldsmid and Crosse, as a great number of dengue sufferers seen by us had not consulted any medical man at all, and these were generally the mild cases which may shew an absence of certain symptoms or signs. Thus, while we agree in the main with them, we would modify their description in certain particulars. We do not think a rash is often entirely absent, but it is often so transitory or slight that unless the patient is under medical examination, and even then at times, it is easily overlooked. This applies not only to the preliminary but to the later rash. Our experimental cases bear this out. It will be seen that in several cases we were unable to make up our minds at all as to whether a rash was or was not present.

The distinction also between the prodromal and later rash is, in our opinion, not very valuable. Although in some cases it is possible to note definite skin eruptions at two periods separated by a period in which the rash is absent or not distinct, there are such great variations in the degree and type of the skin conditions of dengue that the distinction into two rashes is not of great value.

Early in the disease it is unusual to find a definite eruption though we have seen cases with well-marked measly rashes within forty-eight hours of the onset. A hypersensitiveness of the skin which tends to the production of blotchy erythema on points of pressure is an early sign and *tache cérébrale* is well-marked in most cases. The red congested condition of the face has been referred to before. In the early stage it is quite frequent for two observers to differ as to the presence of a rash. The more definite skin eruption is generally found later. Though it may be found well marked from the second day, it may not be noticed till the fifth or seventh day. It presents somewhat variable characteristics and lasts from a day or so to (rarely) several weeks, and is usually followed by slight desquamation and sometimes by intense itching.

We have not sufficient data to describe accurately the distribution of the rash, but we have found it affecting almost any part of the trunk and limbs. It seems as a rule to be less distinct on the face which merely shews congestion. On the back, especially in the lumbar region, it is frequently very distinct and extends round to the abdomen where it is often less apparent. The legs and arms are frequently affected, as a rule the arms shewing more definite lesions. The hands are liable to

be affected, and bright pink spots, followed by intense itching and desquamation, are sometimes found on the palms.

The characteristic of the rash has been described by someone as its "want of characteristic." We think a good definition for the rash in many cases is "mid-way between that of measles and scarlet fever, but less definite." It is, as a rule, some form of a blotchy erythema, though especially in later stages the eruption does not completely fade on pressure. The size, shape and intensity of the blotchings to a great extent account for the differences in appearance. In most of the cases seen by us patches of red skin alternate with pale (normal) patches in a most irregular mottling. The red patches shew no definite point of maximum intensity, but at times the hair follicles are red and prominent giving a strawberry appearance to the red blotches. The red areas do not shew definite lines of demarcation from the adjacent normal skin. The blotchings vary in size but are usually not more than a quarter to half-an-inch square. On the legs of one patient, however, there were large irregular patches much larger than this and of a very bright pink. At the same time this case had a dull measly mottling on the trunk.

In some cases we have seen a very characteristic reddening and swelling of the elbows of a peculiar tint suggestive of a stain of eosin that has been partly washed out. This may be surrounded by a papular condition in the vicinity. Papular rashes have been rarely noted by us, but sometimes are seen on the feet or lower legs.

We have seen no urticarial cases, but these are described by Goldsmid and Crosse and others, and some patients have told us they had had this condition. They present another variation of the skin lesion.

Two other skin conditions should be noted here. In the North Coast district we have seen several cases of a papulo-pustular condition around the ankles extending up the leg for perhaps twelve inches. This was described to us by several patients as a sequel of dengue, but we are not sure whether this was not due to infected mosquito bites or to infection conveyed by scratching the irritable desquamating skin. *Jaundice* is said to occur in some cases and we have seen it in a few ourselves, but it has never been more than slight. It is of interest however in connection with the suggested relationship of dengue to yellow fever.

Diagnosis. From *influenza* the diagnosis rests chiefly upon the absence of coryzal symptoms, usually present with the so-called "influenza" seen in this country. As we have previously mentioned, the absence of coryza is a noticeable feature in dengue. Twenty-six

persons were specifically questioned on this point and all but one denied having any "cold in the head," "running at the nose," etc. Cough, again, which may be a feature of certain influenzal attacks, is usually absent. It was described in only seven out of twenty-four cases questioned. When present it is seldom more than a slight irritative cough, probably associated with the naso-pharyngeal congestion which is often present. The rash, and double phase temperature, and eye pains on movement, are important points when present.

From *scarlet fever and measles* dengue may be difficult to differentiate in isolated cases, and typical cases with a rash occurring early in an epidemic are often diagnosed as measles or scarlet fever. The coryza, nature and distribution of the rash, and Koplik's spots should generally, however, make a diagnosis of measles possible. The pulse in measles is rapid, in dengue often relatively slow. In scarlet fever the early vomiting, throat angina, type of rash, quick pulse and leucocytosis are important points.

The diagnosis from *yellow fever* is not of much importance in this country at present, but should be kept in mind in view of the possibility of the introduction of yellow fever into the *Stegomyia* infested part of Australia. *Jaundice*, though sometimes seen in dengue, is not frequent. Albuminaria is absent in dengue. The slow pulse of yellow fever which is used as a differential sign by Guiteras, cannot be employed with the dengue of Australia. The mild nature of the disease is a practical point when dengue is epidemic though we cannot exclude the possibility of mild cases of yellow fever appearing. In fact some observers have suggested that the dengue of Australia is really a modified yellow fever. The history of the disease and the known variability of dengue in various parts of the world, even in different parts of Australia, and the fixed mild character of the disease here, are arguments against this. It seems probable however that dengue fever is a disease closely related to yellow fever.

Acute rheumatic fever should be considered, but the localisation of pains in the joints and the absence of rash are usually sufficient. Inasmuch as other observers have described painful, hot swellings of the joints in some epidemics of dengue, it is possible that further investigation may disclose such cases in Australia. With one doubtful exception we have never seen any joint affections. This case was a child in the early febrile stage of some infection, who had pains and some swelling in several joints, but we were not able to follow the case further and do not know the final outcome. One of us diagnosed the case as "acute rheumatism."

III. MOSQUITOES AND DENGUE.

(*a*). *Previous work bearing on the experimental production of Dengue Fever by Mosquitoes.*

Graham (1903) reported experiments which are generally regarded as showing that *Culex fatigans* is able to convey the infection of dengue fever. In his experiments, four men slept under mosquito bars containing mosquitoes which had bitten dengue patients. Three cases of dengue resulted, four, five and six days after the first biting. The other case was unsuccessful. These experiments were conducted in a dengue district, and, to obviate the possibility of other means of infection in the infected district, Graham took mosquitoes to a mountain village where no cases of the disease had occurred and similarly infected there two persons, with incubation periods of four and five days respectively.

He admits that in many, perhaps in all, of his experiments *Stegomyia fasciata* were present amongst his mosquitoes. He seems to us, therefore, at most to have proved that *mosquitoes* can carry the disease, the variety or varieties remaining in doubt.

Graham gives also, but without convincing detail, the history of a case injected with the salivary gland of a *Culex*, which had fed on a dengue patient twenty-seven days previously. He says the patient "had a chill on third day and high fever, and an attack resembling in every way that of dengue, but so strong that I desisted from further experiments in that line." "That this was not septicaemia was proved by the finding of numerous dengue parasites in the blood." The second sentence suggests a doubt as to the diagnosis, and the finding of the "dengue parasites," which Graham discovered in his dengue cases, but which were probably artefacts, cannot be regarded as proof of the nature of the disease.

It is interesting to note Graham's remarks as to the distribution of *Culex fatigans* and *Stegomyia fasciata*. These appear to have been both plentifully present in Beyrouth, but on the higher parts *C. fatigans* was the principal mosquito, whilst in some villages there were few or no mosquitoes at all. As far as we can gather from his paper, the distribution of the dengue fever may have corresponded closer with the *Stegomyia* distribution than with the *Culex* distribution, but he has not analysed this point.

Bancroft's (1905) results were no doubt vitiated to some extent by the fact that he was working in an infected district. This may operate

in two ways. Firstly, his apparently successful cases might have acquired the disease in some other fashion, and, secondly, his failures might be due to the cases experimented with having passed through mild attacks of the disease previously. He had two apparently successful cases, the subjects of which were bitten by *S. fasciata* twelve and ten days after these had bitten dengue patients, whilst in the failures the persons bitten were bitten fifteen, fifteen and seventeen days after the mosquitoes had fed on individuals suffering from dengue. His experiments cannot be regarded as in any way conclusive, but are highly suggestive, and one is inclined to wonder that they have apparently not been repeated since. He notes that persons living in the country (non-infected districts?), visiting town friends with dengue in the daytime, acquired the disease, and deduces from this that if dengue is a mosquito-borne disease, *S. fasciata*, which is diurnal in biting habits, may be an efficient agent in the transmission.

Ashburn and Craig (1907) report one successful case in nine persons bitten by *Culex fatigans*. They regard three of these cases as not fair experiments, as proved later by unsuccessful intravenous injections of dengue blood, and another because he had possibly previously had the disease; the other three subsequently developed mild attacks of dengue on inoculation. One person was not bitten by the mosquitoes.

The mosquitoes used had been reared in captivity, and then fed on dengue cases. In the successful case the subject was exposed under nets on September 12th, 1906, to the bites of *C. fatigans*, which had bitten a patient with dengue on September 11th, 1906, but he was not bitten until the night of September 13th, and developed no symptoms until the night of September 17th. His temperature, however, rose on the 16th nearly twenty four hours before. The incubation period would be from three and a half to somewhat over four days. The symptoms appeared to be fairly typical, and there was a slight rash on the abdomen and chest.

It is to be noted that the chart of this case shows the temperature to have been above the normal from the 13th September. This tendency to be above normal may be noticed in several of the charts of injected cases shown by these authors as occurring well before the onset of the fever.

The successful case was probably one of dengue, but arguing on analogy with yellow fever, the very short mosquito "ripening" period (less than two days) would make one accept it with reserve as originating from the mosquitoes. One cannot certainly exclude the possibility of

there being other sources of infection. Failing other evidence, the case is undoubtedly very suggestive of the possibility of *Culex* being a vector of dengue, but we can hardly understand the importance attributed to this isolated case by most text-books.

In reviewing the above series of experiments carried out by observers in three different parts of the world, it will be seen that as regards Graham's observations, whilst the evidence very strongly suggests that *Culex fatigans* is transmitting agent, this cannot be considered as being definitely proved on account of the probability that *Stegomyia fasciata* were included amongst the mosquitoes used.

The results of Ashburn and Craig are much more doubtful from the point of view of incriminating *C. fatigans*. Their mosquitoes apparently conveyed the infection so soon after having bitten a true case of dengue that no reasonable time could have elapsed to enable the organism of dengue to go through a phase of its life cycle in the mosquito. If their successful case arose from the bites of *C. fatigans*, and was not a case of natural infection, the most reasonable view to take is that in this instance the mosquitoes merely acted as infected lancets and not as true intermediate hosts.

Bancroft's experiments on the other hand very strongly support the view that *S. fasciata* transmits the disease, and are only vitiated by the fact that the experiments were conducted within the endemic and epidemic area.

REFERENCES.

ASHBURN and CRAIG (1907). Experimental Investigations Regarding the Etiology of Dengue Fever. *Phillip. Journ. of Sci.* II. 93.

BANCROFT (I. 1906). On the Aetiology of Dengue Fever. *Aust. Med. Gaz.* p. 17.

GRAHAM (1. VII. 1903). The Dengue: A Study of its Pathology and Mode of Propagation. *Journ. of Trop. Med.* p. 209.

A few further references not, however, of much importance will be found in Sticker, *op. cit.* [Editor.]

(*b*) *Australian mosquitoes as conveyers of disease.*

As both *Culex fatigans* and *Stegomyia fasciata* are common household pests in most parts of Australia which have suffered from this recent epidemic of dengue, it seemed quite probable that, if a mosquito were a vector of this disease, it might be one or other or both of these two species. *C. fatigans* is common in summer time in the southern districts of Australia where dengue does not occur, whilst the distribution of the disease is practically that of *S. fasciata*. Coupling these facts with the

observations and experiments of Dr Bancroft, greater suspicion naturally falls upon *S. fasciata* than upon *C. fatigans*. In our experiments both of these species were used. *S. fasciata* was found to bite freely in captivity in the day-time, but *C. fatigans*, though it did bite at night-time, was more shy and difficult to handle. In considering the transmission of the disease, a study of the habits of the mosquitoes in an infected area is important. Observations of the mosquitoes in general will show why it is that some species can readily transmit disease, whilst in the case of others disease transmission is unlikely. *C. fatigans* and *S. fasciata* are essentially domestic mosquitoes, thereby possessing increased facilities for transmitting diseases to human beings over "wild" mosquitoes. It is highly probable that both are introductions to Australia, having been non-existent here before the arrival of the white population. Both can apparently be easily conveyed from place to place by means of human agencies.

In this place it may be well to review shortly a few of the Australian mosquitoes which may play a part in conveying disease, or are present in exceptional numbers.

Culex fatigans Wied. This is the common domestic mosquito and is probably almost universally distributed throughout Australia. One of us (J. B. C.) has met with it abundantly in Sydney and in many country towns in New South Wales, and also in Adelaide. In the warmer parts of Australia it may probably be found biting throughout the year, but in the southern parts it disappears throughout the cold months, though during warmer evenings an occasional individual may be met with. It is essentially a night-biter and a feeder in the dark. We have never met with it biting during the day-time, but it occasionally bites in the evening in a poorly lighted room. Under these circumstances, it is more especially the legs or some other portion which is not exposed to the light that are bitten. The hum of the mosquito at night-time is very disturbing, the anticipation being more annoying than the bite itself, which in the cases of a number of individuals can hardly be noticed. Many of those bitten by the mosquito do not react by the raising of a wheal. At any time in bed the approach of the mosquito can usually be felt by the currents of air produced by the wings. This draws attention to the part where the mosquito settles, and, as it begins to feed, in many cases a slight but indefinite pricking sensation indicates the exact site. However quickly the hand is raised without disturbing the bedclothes, it is only rarely the movement is sufficiently quick to enable the mosquito to be destroyed. Its breeding place is in various

domestic water supplies—probably the cisterns of water closets may prove to be one of the most important of these. In places such as Sydney where during summer in some seasons there may be long periods without any rainfall, and in other seasons abundant rains for many days, the number of mosquitoes may be greatly increased under the latter conditions, indicating that breeding places form as a result of collections of rain-water. It has not yet been ascertained exactly where these outside breeding places are located in a city like Sydney, where, in the better residential localities, tins, broken bottles, and similar receptacles are not left lying about; but it is probable that places such as depressions in gutter-spoutings are some of the most important sites.

The distribution of this mosquito extends far beyond the areas in which dengue fever has occurred. For instance, though the mosquito is abundant in the neighbourhood of Sydney, no indigenous cases of dengue are known to have arisen in this city. Considering that imported cases of dengue have been not uncommon, the inference might be drawn that if *Culex fatigans* were capable of transmitting this disease, endemic cases should in consequence have arisen in Sydney.

Stegomyia fasciata Fabr. This species occurs in Queensland and extends into the northern coast towns of New South Wales. We have found it at Tweed Heads, Murwillumbah, Mullumbimby, Byron Bay, Casino and Grafton. Dr Ferguson has also identified specimens from Maclean and Tabulam.

Though the species has been recorded from Newcastle and from Victoria, there seems considerable doubt as to the identification, and in all probability specimens so designated were really *Scutomyia notoscripta*.

The insect is a day-biter, and during the recent dengue epidemic it was abundant in houses in the affected district, usually being more active in rooms that were dimly lighted. It was found breeding in water tanks, and in similar domestic supplies, one such source worthy of notice being open water in connection with acetylene gas installations. It is interesting to note that larvae were drawn off from the bottom of a tank which was four to five feet high, and which, as heavy rain had been falling for some days, was presumably full of water. In two or three jugs of water drawn off from the bottom, some larvae were obtained which afterwards hatched out.

We were able to confirm the statement that the eggs of *Stegomyia fasciata* can resist drying for some while and then develop under suitable conditions. In our second batch of these mosquitoes a number of eggs were laid in a small dish of water. On June 29th this dish had become

perfectly dry and was left exposed on a laboratory bench until August 30th, that is, during the end of winter and the beginning of spring. It was then immersed in water and in a few days some of the eggs hatched. Owing to the weather being cold the larvae developed very slowly, but early in November an adult which had recently emerged from its pupal stage was found floating on the surface of the water. At this period it was also noticed that a number of further eggs had hatched, the weather having become warmer. It would seem therefore that not only did the eggs resist two months absolute drying and then develop immediately on immersion in water, but that they also remained for some weeks without developing in this water until the weather became warmer.

A review of the above distribution of *Stegomyia fasciata* will show that the recent epidemic of dengue was nearly co-extensive with it. Thus, the epidemic appeared in all of the towns mentioned with the exception of Maclean and Tabulam, about which we have no information. It is interesting further to note that the epidemic extended southwards along the railway line, and this is doubtless explained by travellers becoming infected in one town and developing the disease in another, and there infecting the local mosquitoes and starting a fresh centre of the infection. Probably railway communication also facilitates the dispersal of *S. fasciata*. Though we did not find *S. fasciata* in railway carriages at Murwillumbah during the epidemic, we found them in the Station-master's office at Byron Bay. At Murwillumbah the mosquitoes found in the railway carriages were chiefly *Culex fatigans* and occasionally *Culicelsa annulirostris*.

Scutomyia notoscripta Skuse. This is a widely distributed mosquito throughout Australia, though we have never met with it in much abundance. It resembles very closely *Stegomyia fasciata* in its thoracic markings, but can be at once distinguished by a pure white band on the proboscis. It may occasionally be found biting inside houses. It is not known to be responsible for conveying any disease to human beings.

Culicelsa vigilax Skuse. This is the common bush mosquito so numerous at certain periods of the year in the neighbourhood of Sydney and other similarly situated districts. In places it is exceedingly numerous, as for instance in some of the creeks running into the Hawkesbury River where human beings may be attacked by hundreds of these insects at a time. The bite is rather painful and often raises small wheals. It is very interesting to compare the behaviour of this mosquito when attacking man with that of such domestic mosquitoes as *Culex fatigans* or *Stegomyia fasciata*. The two latter are exceedingly wary in their

habits, so that it is a matter of skill to kill them when they are attempting to bite. With *Culicelsa vigilax*, however, the insects settle on the hand or face and the finger can be slowly lowered down upon them and can crush them without disturbing them. *C. vigilax* is an Australian species accustomed to live in our bush, and probably to feed chiefly upon birds and marsupial hosts, which are unable to protect themselves against attacks of the mosquitoes by slapping them with hands.

Culicelsa annulirostris Skuse. Though a widely distributed mosquito in Australia and present in the area affected by the recent epidemic of dengue, there seems no reason at present to consider that it is responsible for the conveyance of any disease in man.

Nyssorhynchus annulipes Walker. This mosquito, the chief malarial transmitter in Australia, appears to be widely distributed throughout the continent, but in the southern parts as a rule only in small numbers. Here and there areas exist where it is present in sufficient numbers to be a source of danger should imported malarial cases reside there. In the coastal parts of Queensland, however, and in the Northern Territory, its incidence is sufficiently great to maintain in places endemic foci of malaria.

As regards the diseases spread or possibly spread by mosquitoes in Australia, malaria has already been mentioned. Our experiments on the conveyance of dengue have clearly proved that *Stegomyia fasciata* is responsible—is perhaps alone responsible—for the spread of this disease in Australia. The same mosquito, as is well known, is the transmitting agent of Yellow Fever. *Culex fatigans*, the common domestic mosquito, is a transmitting agent of *Filaria bancrofti*, and is responsible for the distribution of this disease in Queensland. So far there are no other diseases of human beings in Australia which are known to be transmitted by mosquitoes.

IV. REVIEW OF THE RESULTS OF THE MOSQUITO EXPERIMENTS.

A. *First Series of Mosquito Experiments.*

Details of our first series of mosquito experiments will be found in Appendix II. Four cases were bitten by *Stegomyia fasciata* and two by *Culex fatigans*. Negative results were obtained. There was, however, a very large mortality among the mosquitoes collected, and the bitings, except in one case, which received ten bites, were unsatisfactory.

B. *Second Series of Mosquito Experiments.*

In our second series of experiments mosquitoes were collected in Mullumbimby and the surrounding district, about 100 *S. fasciata* and 112 *C. fatigans* being thus obtained. The insects were collected from the hotel at which we stayed at Mullumbimby, from the post office, and from private houses in the town and district in which dengue fever cases had occurred—in some cases from the actual bedroom where patients were lying sick with the disease. A few mosquitoes were caught on the journey from Brisbane to Mullumbimby.

Occasionally *Culicelsa annulirostris* was found in Mullumbimby, and on our journey, but is not included in the above, and, with the exception noted in our *Culex fatigans* results, was not used in our experiments.

The *Stegomyia fasciata* and *Culex fatigans* were transferred to special cages, one containing *S. fasciata*, the other *C. fatigans*. The cages were made with a rounded opening, to which was attached a net sleeve. Through this the hand could be passed to add freshly caught mosquitoes and for the biting experiments.

At Mullumbimby, on May 8th, 1916, a dengue patient (*X*), who became ill on May 7th, was bitten by the *Stegomyia* then in the cage; on the 9th, he was again bitten by *Stegomyia*, and on the evening of the 8th, he was bitten by *Culex*. Both species of mosquitoes bit this patient well, and thus many of the Mullumbimby district mosquitoes had certainly been fed on the blood of a dengue case in the acute stage. Exactly how many mosquitoes bit this patient it is impossible to say, as this part of the work was conducted in a badly-lit bedroom.

On May 11th, 1916, these mosquitoes arrived in Sydney.

Biting experiments with the *Stegomyia* were conducted on May 11th, 12th, 13th, and 14th, and with *Culex* on May 11th, 12th, 13th, and 14th, as shewn more clearly later.

On May 15th, some seven *Culex* and eleven *Stegomyia* collected in the Grafton district, chiefly from houses of dengue patients, were added. Over 112 mosquitoes were collected in Grafton, of the following species: *Stegomyia fasciata*, twenty seven; *Culex fatigans*, forty six; *Culicelsa annulirostris*, thirty seven; *Nyssorhynchus annulipes*, two. But although all care was taken, the mortality between Grafton and Sydney was large, and hence only this small number was added to the boxes.

Further feeding experiments were made with the remaining mixed Mullumbimby and Grafton mosquitoes, viz. with *S. fasciata* on May 15th,

16th, 17th, 18th, 19th, and 23rd: and with *C. fatigans* on May 15th and 16th.

On June 29th, the cages used in the experiments were emptied of the dead mosquitoes, and the remaining bodies that were not crushed were examined separately with a hand lens. Seventy-five *Stegomyia* were counted (two being males) in the *Stegomyia* cage; no other mosquitoes were found in this cage. Seventy-eight *C. fatigans* (two being males) and one *Culex annulirostris* were found in the *Culex* cage.

This procedure forms an additional check by another observer (J. B. C.) that the classification of mosquitoes was made accurately by B. B., and although about twenty-five *Stegomyia* were unaccounted for —probably they had been unrecognisably crushed in travelling, etc.— we can be reasonably certain that no *Culex* was included in the *Stegomyia* cage with which we obtained our positive results.

Summary of Experiments.

The following is a short summary of the experiments made and results obtained with the mosquitoes, taking the nine persons volunteering *seriatim*:

Case I. J. G., male, laboratory assistant (18 years), the subject of an unsuccessful *Stegomyia* biting experiment of the first series, was bitten on May 11th, 1916, at 2.15 p.m. by some twenty-eight *Stegomyia*. He remained well until the afternoon of the 19th, eight days later, when he noticed he had headache. That evening at 7 p.m. (eight days and five hours) he was again bitten by *Stegomyia*, and, while sitting with his hand in the cage, first became definitely ill. He passed through a typical attack of dengue fever, shewing a double temperature curve, rash, and symptoms described in detail below. Blood from this case reproduced the disease on injection. *Result positive.*

Case II. McD., male, laboratory assistant, not previously the subject of experiment, was bitten on May 12th, 1916, by ten *Stegomyia* and on the 18th by three or four *Stegomyia*. He remained well until June 3rd, seventeen days from the second biting and twenty-two days from the first biting, when he had an influenzal attack with coryza for a few days, with no rash and nothing suggestive of dengue. *Result negative.*

Case III. G., male, laboratory assistant, not previously the subject of experiment, bitten by about nine *Stegomyia* on May 13th, 1916, and by about three *Stegomyia* on the 17th. No symptoms have followed these bitings to date—July 14th, 1916. *Result negative.*

Case IV. *Wm.*, male, laboratory assistant, not previously the subject of experiment, was bitten by about thirty-six *Stegomyia* on May 14th, 1916 (mid-day), and by about thirty-six *Stegomyia* on the 15th (12.30 p.m. and 4.30 p.m.). On the 20th (six days and nine hours from first biting), whilst going to bed at night, he became ill and had a typical attack of dengue, with double temperature, rash and other symptoms detailed in Appendix III. His blood on injection reproduced the disease. *Result positive.*

Case V. *M.*, female, a nurse, was bitten by eighteen *Stegomyia* on May 16th, 1916 (noon) and became ill on the 25th, 10 p.m. (nine days ten hours later), and passed through a rather severe type of dengue with marked rash and double temperature. No blood was taken from this case for injection experiments. *Result positive.*

Case VI. *B. B.*, medical practitioner, was in dengue fever districts — Mullumbimby, Casino and Grafton—leaving Grafton for Sydney by boat on May 13th, 1916. To keep the mixed Grafton mosquitoes alive, he allowed them to bite him on the 12th and 14th, but remained perfectly well till the 23rd (2 p.m.); ten days after leaving the dengue district, he was bitten by fifteen *Stegomyia*. He remained well till May 29th, and his temperature was normal till May 31st, on rising at 9 a.m. (seven days and nineteen hours), he then became definitely ill and passed through a severe attack of dengue, with definite prodromal and secondary rashes, double temperature, and marked pains, etc., as described in Appendix III. Blood from this case reproduced the disease on injection. *Result positive*, but open to criticism since *B. B.* had been in a dengue district eighteen days before the attack developed.

Case VII. *W. T.*, bitten by one *Stegomyia* on May 12th, 1916. No illness followed. *Result negative.*

Case VIII. *M.*, a patient at a hospital, was bitten by *Culex fatigans* as follows: on May 11th, 1916, by about twelve; on May 12th and 13th by an unknown number; on May 14th, by at least twenty. *Result negative.*

Case IX. *J. O. S.*, laboratory assistant, a subject of *Culex* experiment in first series, was bitten by *C. fatigans* as follows: on May 15th, 1916, by two; on May 18th, by an unrecorded number. No symptoms followed. *Result negative.*

Discussion of Results.

In discussing the above results, it is important for the reader to bear in mind that our main object was to determine whether either or both of the mosquitoes experimented with were capable of transmitting

infection. We were quite in the dark, even if one or both species of mosquito were a transmitter of the disease, about a number of other important circumstances connected with such a possible means of transmission. The mosquito, if it carried infection at all, might or might not need a period to elapse after biting a patient before it became able to infect another person, and might remain infective for a period quite undetermined by us. Hence mosquitoes collected might not prove successful transmitters, not because they could not carry infection, but because they were not for one reason or another "ripe." Therefore, although our mosquitoes were collected in a district where dengue was prevalent, some from houses where patients were actually ill, and many from houses where patients had recently been ill, we felt it advisable to increase the chance of getting results by letting them bite patient *X.*, who had acquired the disease in the usual manner, on the dates mentioned. We were not in a position, and did not try, to solve the question of the "ripening" period, if any, nor of the period during which the mosquitoes remained infective.

Again, we deemed it advisable to have our first volunteers bitten more than once, and that because of the uncertainty as to whether the mosquitoes had "ripened," especially if infected from the known bitten patient, and because of the unknown time which the mosquitoes might remain infective. We foresaw that, to a certain extent, these multiple bitings might complicate our results and prevent us from obtaining the exact incubation period, but we attempted to arrange the experiments in such a way that we might hope to elucidate this point. As it turned out, the double biting has actually only interfered with the understanding of the incubation period in one case (*Wm.*), and the later volunteers, being only bitten once, tend to confirm, in this case, the longer incubation period rather than the five days five hours period which may have been the incubation period for *Wm.*

That we have succeeded in proving the principal hypothesis, the possibility of transmission by mosquitoes, depends mainly on the satisfactory nature of the evidence that our apparently successful cases were really instances of dengue fever. If we are successful in this there seems no escape from the conclusion that transmission had occurred through the agency of the *Stegomyia fasciata* used by us.

We have not, in our opinion, shewn conclusively that *Culex fatigans* may not also spread the disease, though we think this unlikely.

That the disease which followed the biting of our volunteers by *Stegomyia* was dengue there can be no reasonable doubt when the

following circumstances are considered. In the four successful cases the illness began at a period of from six (possibly five) to nine days after being bitten, an incubation period the limits of which were the same as those of cases of dengue fever conveyed by blood inoculation from previous cases. The symptoms, signs and clinical characteristics of the disease in the four successful mosquito cases were indistinguishable from those in attacks of dengue naturally contracted. The rashes in two of the cases were typical of those seen in certain dengue cases, and could not be confounded with those of measles or scarlet fever, the other febrile complaints with which a distinct rash is usually associated. After the rashes disappeared, these two patients suffered from intense itching of the parts which had been affected by the rash, in one case to such an extent as to be almost unbearable. Such intense pruritus, rendering life temporarily a burden, has occurred in some instances in the North Coast district of this State following the disappearance of the rash of dengue. This itching, following a febrile complaint accompanied by a rash, we consider to be almost pathognomonic, when it occurs, of dengue.

Of other noteworthy features characteristic in general of attacks of dengue fever, the following may be noted. All the four patients shewed a swollen, hot-looking condition of the face, with a flushed, red suffusion, resembling somewhat that seen in the incipient stages of measles, or after an alcoholic bout. They all had, in fact, what may be called the "dengue face." All the cases shewed a sudden onset, more or less characteristic of dengue, and not so common in other febriculae. In all there was a distinct tendency to a double rise of temperature, the early rise being followed by a fall for a few days and then by a final rise. The blood examinations made during the course of the disease shewed a definite leucopenia, a characteristic feature of dengue.

In the three cases in which blood was taken during the height of the disease and injected into volunteers who had never been in contact with the patients, the disease was successfully transmitted after the usual incubation period.

From the above summary it is clear that the disease in the four volunteers was not measles, German measles, scarlet fever, or any of the other acute infective fevers accompanied occasionally by similar rashes, such as the early stage of small-pox.

In all large communities there are continually present febrile complaints not accompanied by definite rashes, which are loosely styled "influenza." These vary much from time to time, and probably represent a number of distinct entities, with features so little characteristic,

and symptoms so mild and evanescent, that it has not been possible as yet to differentiate one from another. Many of these are accompanied by a definite coryza, which was absent in our experimental cases. During the course of our experiments, such complaints were not absent from Sydney, and though in specific instances individual cases might resemble aberrant cases of natural dengue, none could be considered as typical cases, such as were our volunteers, and rashes did not develop.

Having established that the disease occurring in our four volunteers was dengue fever, it is necessary to shew beyond reasonable doubt that the disease developed as a result of the bites of certain infected mosquitoes, *Stegomyia fasciata*. As two of the four individuals had never been in a dengue area, while a third had been away from such an area for eight years, and as the experimental bitings took place in a district in which dengue is unknown, except as imported cases, and as we know of no other means by which they could have become infected, no other conclusion is left save that the *Stegomyia* transmitted the disease. It is true that one of us, who contracted the disease naturally, had been in more or less daily association with two of these three volunteers, and had seen the third for a few minutes, but it is hardly reasonable to suppose that he should have carried infection to these three persons and to these three only, and yet have failed to convey infection to other members of the staff and to his own household. The fourth volunteer, one of us (*B. B.*), had returned recently from a dengue area. It might, therefore, be suggested that the disease from which he suffered was naturally contracted there. It will be noted, however, that he had been away from the dengue area for a period of time far exceeding the established limits of the incubation period, so that, were his case one of natural infection, then the incubation period, in his case, of a typical attack must be considered to be twice as long as our results in other cases would indicate.

As further shewing that the disease developed by the four volunteers is to be attributed to the bitings of the mosquitoes, is the fact that, though each volunteer was bitten on different days and with varying intervals between them, the incubation periods of their complaints fell within the time found to be the incubation period in our blood inoculation experiments. Such results in four instances must be considered more than mere coincidences.

That we were not successful in conveying the disease to all the volunteers is not to be wondered at. These other persons were certainly not so extensively bitten as were the successful cases. As

perhaps only a certain number of mosquitoes were infective, and as mosquitoes engorged with blood one day, whether infective or not, may not feed again perhaps for several days, it can be understood how such failures can occur, whilst the opinion that there may be a possible minimum amount of infective material necessary to ensure successful inoculation by bites of the mosquitoes is another hypothetical explanation.

Apart from this, the positive results obtained in the four successful cases overshadow entirely the three negative results, which need only be considered from the theoretical point of view as to why the patients did not develop the disease, and not from the practical point of view, as to whether or not *Stegomyia* is the vector.

Incubation Period. We are able, fortunately, to draw reasonably accurate conclusions, even from the first doubly bitten cases, as to the incubation period.

Case I. J. G. became ill while actually being bitten for the second time. His is obviously an eight days' incubation period. *Case IV. Wm.*, the second successful case, was bitten on two successive days, and his incubation period would be six days and nine hours, or five days five hours, depending on whether we count from his first or second biting. In the case of *Nurse M.* the incubation period is definitely nine days ten hours, and in the case of *B. B.* about seven and three-quarter days, if we count from the first rise of temperature, and about five and three-quarter days if we count from the first feeling of malaise.

This gives us for our mosquito cases an incubation period of approximately six to nine and a half days, possibly five and a quarter to nine and a half days.

General Conclusions from Series II.

(1) *Stegomyia fasciata* caught in a dengue infected district in the surroundings of cases of the disease, some of them known to have fed on a dengue patient on the first and second days of his illness, transported to a non dengue district, reproduced the disease in four out of seven persons on whom biting experiments were conducted.

(2) Blood taken from three of these four cases reproduced the disease when injected into further persons. The blood of one case was not tested.

(3) The incubation period of the four cases was found to be possibly between five and nine and a half days, probably between six and a half and nine and a half days, counting from the biting to the definite onset.

(4) No known case of contagion occurred from any of the above four cases.

(5) No evidence was obtained from two cases, one of which was heavily and repeatedly bitten with *Culex fatigans*, that this mosquito can transmit dengue fever.

V. Review of the Results of the Inoculation and Allied Experiments.

(*a*) *Clinical description of cases artificially inoculated in Sydney.*

These observations on the clinical phenomena of Dengue Fever are based on the results of thirty-two experimental inoculations, etc., for the transmission of the disease, made at the Rookwood State Hospital and Asylum. Thirty patients who volunteered for the experiments were treated in various ways.

Of the twenty-eight *inoculations* thirteen experiments gave positive results, the patients developing what we regard as undoubted dengue fever; twelve experiments gave definitely negative results; and seven gave doubtful results. We discuss the doubtful and negative cases elsewhere. In many of these the nature of the experiment led one to expect a negative result. The clinical description of the thirteen successful cases may be discussed in detail.

The incubation period is reckoned as the time elapsing between the date of inoculation and the appearance of the initial symptoms or sign. Omitting four positive cases which had more than one injection and in which the incubation period is not quite definitely established, the other nine positive cases gave the following results: five to six days—three cases; six to seven days—two cases; seven to eight days—one case; eight to nine days—three cases. Hence the incubation period ranged from five to nine days. Five gave periods between five and seven days.

The onset was usually sudden, the symptoms, at first mild, becoming well-defined within a few hours. The most consistent initial symptom was *headache*, usually occipital, less often frontal, rarely general. In only one case was headache absent. A few had dizziness and most complained of a "shivery feeling" in the early stages.

The temperature rose fairly rapidly from the beginning. On one occasion the temperature, and not the headache was the earliest sign of infection: whilst in four cases the temperature and headache were

practically coincident in time of appearance. Usually the temperature rose so rapidly that the maximum was attained on the first or second day. The temperature curve shewed some degree of fluctuation with a rapid subsidence, the fall being practically by crisis. Two of the cases shewed the typical diphasic temperature charts. Four shewed irregular diphasic charts; four shewed irregular charts; and two shewed definitely monophasic charts. One case relapsed and shewed a monophasic variation in the first attack and a diphasic variation in the relapse. The highest temperature recorded was 104° F. (average 102°–103°).

The pulse rates in the inoculated cases form a very interesting study, but we wish to make clear from the start that our records are open to criticism from several points. Most of our cases were men close to or over 50 years of age, and all were inmates of an Asylum and may thus be regarded as of a selected type, and these may normally have pulse rates different from the normal active man of the outside world. Again, we have only the pulse record *after* the onset of the illness, and have not been able to control such pulse rates with the rates before the injection or other exhibition of presumably infectious material. We therefore present our own results with due reserve. We are inclined to think from some of the records of doubtful or negative cases that some degree of absolute bradycardia may be a feature of the pulse in such type of persons. We have submitted thirteen cases which we regard as having suffered from experimental dengue to detailed analysis, and have analysed the pulse-temperature ratio—(1) in the first stage of the fever; (2) in the second stage of the fever (in cases where the diphasic temperature variation was not clear we have made observations in early and later stages of the febrile phase); and (3) in the post febrile condition. In Case 25 which we regard as an instance of relapse, the periods analysed were in the first monophasic febrile phase, in the inter-febrile interval, in the first and second stage of the relapse, and in the post-febrile phase. Summarising these results as well as possible in these thirteen cases we may say that:

1. Eleven out of the thirteen shewed during the febrile stage or stages marked *relative bradycardia*. One case shewed periods of *definite absolute bradycardia*.

2. In what may be roughly regarded as the first febrile paroxysm, five cases shewed marked *relative bradycardia*, and seven shewed slight *relative bradycardia* (in one case there was no record).

3. In the second febrile stage or in the latter part of an irregular febrile attack, nine cases shewed marked *relative bradycardia*; two shewed

slight *relative bradycardia*, and in two definitely monophasic charts there is of course no record.

4. In the post febrile phase there was marked *absolute bradycardia* in one case (pulse going as low as 42); *definite absolute bradycardia* (pulses at times below 50–55) in six cases; *slight absolute bradycardia* (below 50–60) in four cases; normal pulse in one case and no record in one case.

With the reservations above mentioned it appears from our results (refer to table overleaf) that:

1. There is a tendency to slow pulse in our infected cases of dengue which may manifest itself by absolute or relative bradycardia.

2. Relative bradycardia is a remarkable feature of the latter part of the febrile paroxysm, or of the second phase when it occurs. It may occur from the onset of the fever but is usually in the early stages less marked.

3. Absolute bradycardia is found in many cases in the post-febrile stage. It is generally intermittent in type and alternates with normal or quick pulse rates. It may be also found during the febrile stage.

The appearance of the patient was at once suggestive. The face was flushed, with a suggestion of puffiness about the features, and the conjunctivae were injected. The injection of the eyes and of the pharynx was constant. The facies is comparable to that of a man in the early excitement of alcoholic indulgence, and is not unlike the facies of measles. The patients looked bright and alert, and made little or no complaint even in the cases shewing marked reactions and notwithstanding that no analgesic or antipyretic drugs were used. Although the type of patients volunteering for this work would perhaps be less likely to complain than those of a finer mould, this comparative indifference to the disease is worthy of record, seeing that in the Murwillumbah epidemic the observers describe the facial expression as one of suffering and state that it was necessary in a few cases to resort to morphine. We should, however, correlate the mildness of the symptoms in our experimental cases with the fact that as soon as the patient was found to be febrile he was put to bed and kept there till the attack was over, whereas in many of the severe natural cases seen by us the patients had for a while attempted to carry on their work.

Pain in these cases was not an important feature. The pains complained of were attributed in most cases to "aching muscles" and the common sites were the lumbar region, nape of the neck and limbs. Two subjects complained of "rheumatic pains" but the joints were neither

The Pulse in Experimental (Injected) Dengue.

Review of the Pulse Variation in the Inoculated Cases.

Case	Age	Type	Pulse or febrile phase: 1st	Pulse or febrile phase: 2nd	Post-febrile phase
4	46	Irregular	Irregular marked relative and definite absolute		Definite absolute irregular
6	48	Diphasic	Sl.R.	M.R.	Normal
7	49	Diphasic*	Sl.R.	M.R.	Definite
9	56	Diphasic	(No record)	M.R.	No record
11	—	Monophasic	M.R.	—	Slight
12	47	Diphasic*	M.R.	M.R.	Marked A
13	44	Irregular	Sl.R.	M.R.	Definite
16	55	Diphasic*	Sl.R.	Sl.R.	Slight
17	38	Irregular	Irregular slight relative		Definite
25	52	Relapsing			
		1. Monophasic 2. Diphasic	Sl.R. Sl.R. } Sl.R.	M.R.	Slight
26	67	Monophasic	M.R.	—	Definite
27	50	Irregular	M.R.	M.R.	Definite
29	46	Diphasic*	Sl.	M.R.	Slight

Summary:

		Relative bradycardia				Absolute bradycardia	
Typical Diphasic	2	Slight	7	Marked	9	Normal	1
Irregular Diphasic	4	Marked	5	Slight	2	Slight	4
Relapsing	1	No record	1	No record (Monophasic)	2	Definite	6
		Absolute bradycardia					
Monophasic	2					Marked	1
Irregular	4	Irregular	1			No record	1

S.R. = Slight relative bradycardia. M.R. = Marked relative bradycardia.
M.A. = Marked absolute bradycardia. * = Irregular.

swollen nor tender to touch. Two patients suffered no pain at all. In one or two cases the pains dominated the picture, but even then they were not severe, making the patient unhappy merely for a day or two.

The appetite was usually good and unimpaired. Only one patient complained of anorexia.

Five patients suffered from *nocturnal sweating*. The sweating was generally profuse. These sweats usually recurred for a few nights. One of us (*W. McD.*) records that they bore no relation to the temperature, coming on quite independently of its rise or fall, but examination of some of the charts suggests a relation between sweating and abrupt temperature falls.

Most of the patients slept well, but in four cases *sleeplessness* was complained of on occasional nights.

The rash. Some degree of rash was an almost constant feature, being absent in only one of the positive cases and doubtful in two cases.

The prodromal rash was sometimes difficult to detect. In seven cases some more or less definite rash or eruption was noted. *The later eruption* was found in ten cases and absent in three cases. We have recorded the first appearance on days from the second to the seventh day of the disease, usually on the third or fourth day. The rash lasted a variable time being often visible ten days or more after its appearance. In many cases it is difficult to distinguish between the preliminary and later rashes, and the distinction does not seem a very useful one. Out of the thirteen cases considered the later rash was very distinct in four cases, definite in four cases, slight in two cases, and negative in three cases.

Character of later rash. In some cases the rash was polymorphous. In two cases it was morbilliform. Most commonly it was a pinkish erythematous mottling with irregularly shaped areas (sometimes definitely raised), of varying intensity of colour, surrounding islets of white. In one or two cases the rash covered the whole body, being apparent even on the soles of the feet: but in the main the distribution favoured the upper portion of the body, viz., the back, chest, abdomen and upper arms. Itchiness was sometimes complained of. A constant sign was a congested erythema of the back. This was present in cases shewing no rash. In a few cases the rash persisted and was still visible when the patients were discharged.

Enlarged lymph-glands were palpable in two patients.

Vomiting in the early stages of the disease was observed in two patients. Four patients complained of a cough without expectoration.

The urine analysis gave fairly constant results. The specific gravity varied from 1010 to 1025; the reaction was acid and there was no albumen. In two cases the specific gravity rose suddenly during two days to 1030 and a reduction of Fehling's Solution took place. Following on this, the specific gravity of the two urines fell to 1006 and 1017 respectively, and no glycosuria was detected. Albumen was absent in every case. In this respect, the disease may be contrasted with Yellow Fever, in which albuminuria is so distinctive a feature.

The duration of the disease ranged from four to seven days, though most of the cases were kept in bed for ten days, and the rash persisted at times for two to three weeks. Convalescence was rather protracted and a number of the patients complained of weakness persisting for some time.

The diagnosis would be readily made in an epidemic but would admit of some difficulty in sporadic cases. The sudden onset with headache; the flushing of the face and injection of the eyes and pharynx; the congested erythematous appearance of the back; the occurrence of the rash; and the condition of the pulse—all unite to form a more or less typical picture. Perhaps it would be necessary to differentiate the condition from Influenza, Fibrositis, and Measles.

In all the cases recorded above, the toxaemia would appear to have been slight, as, although the symptoms were well-marked, there was never any anxiety as to the ultimate complete recovery of the patient.

No case of infection occurred other than amongst the artificially inoculated.

Incidence of Symptoms and Signs in the Thirteen Positive Cases.

Headache ...		11
Vomiting ...		2
Cough ...		4
Sleeplessness ...		4
Aching eyes ...		5
Rash	Preliminary ...	5
	Terminal ...	10
Flushing of face...		12
Relative Bradycardia ...		13
Absolute Bradycardia ...		11
Typical Diphasic temperature chart ...		2
Irregular Diphasic temperature chart ...		4
Monophasic temperature chart ...		2
Irregular temperature chart ...		4
Relapsing temperature chart ...		1
Sweating ...		5
Pains	Aching muscle pains ...	8
	Joint pains ...	2
Anorexia ...		1
Marked weakness during convalescence—Several cases.		

(*b*) *Consideration of Cases* 1 *to* 9.

These cases, with the exception of Case 1, all received two injections of material separated by an interval of four days. Case 1 received only the first injection, but it is considered with these other cases because the material was the same as that used for the first injection in some of the other cases.

At the beginning of our work in connection with the experimental transmission of dengue from one case to another our efforts were at

Table shewing incidence, date of appearance, etc., of rash.

		First examination			Later rash				
Case no.	Date of onset	Date	Day	Rash	Date	Day	Duration	Intensity	Remarks
4	19 . iv . 16	20 . iv . 16	2	+ ?	+	–	+	Neg.	Doubtful early erythema. No later rash.
6	16 . iv . 16	17 . iv . 16	2	Neg.	18–19 . iv . 16	3, 4	11	+S	Prodromal rash not noted. Slight later rash.
7	16 . iv . 16	17 . iv . 16	2	+S	18 . iv . 16	3	6	+	Slight prodromal rash. Definite later rash.
9	19 . iv . 16	20 . iv . 16	2	+ ?	25 . iv . 16	7	7	+ +	Doubtful prodromal rash. Marked later rash.
11	23 . iv . 16	23 . iv . 16	1	Neg.	24 . iv . 16	2	11	+ +	No prodromal rash. Marked later rash.
12	25 . iv . 16	25 . iv . 16	1	+	27 . iv . 16	3	17	+ +	Definite prodromal and marked later rash.
13	25 . iv . 16	25 . iv . 16	1	+S	27 . iv . 16	3	11	+	Slight prodromal and definite later rash.
16	3 . v . 16	3 . v . 16	1	+ ?	+	–	+	Neg.	Doubtful early erythema. No later rash.
17	3 . v . 16	3 . v . 16	1	Neg.	7 . v . 16	5	5	+	No prodromal rash. Definite later rash.
25	21 . v . 16	22 . v . 16	2	Neg.	23–26 . v . 16	3, 6	11	+	No prodromal rash. Definite later rash.
26 (32)	11 . vi . 16	11 . vi . 16	1	Neg.	13 . vi . 16	3	13	+S	No prodromal rash. Slight later rash.
27	1 . vi . 16	2 . vi . 16	2	Neg.	+	–	+	Neg.	No rash noted.
29	30 . v . 16	31 . v . 16	2	+ ?	1 . vi . 16	3	15	+ +	Doubtful early erythema. Marked later rash.

Day = Day of Disease. + + = Marked. + = Definite. +S = Slight. Neg. = Negative. + ? = Doubtful.

first directed to establishing in Sydney transmitted cases of the disease by some means or other. Nine volunteers were secured who for a consideration submitted themselves on April 8th, 1916, to injections of material from the bloods of two natural cases of the disease, which had occurred at Murwillumbah. Two or three days later the occurrence of a natural case of the disease at the Coast Hospital, in which the infection had been contracted in the North Coast district, gave us a further opportunity of obtaining infective material, though in this case the patient was convalescing, being in the eighth day of the disease. Material from this second source was injected on April 12th, 1916, into eight of the nine volunteers who had received the first injection four days previously. As the results shewed it is to be regretted that in any case one individual received two separate injections within such a short interval of time. The difficulty of obtaining volunteers together with the desire on our part at this stage of our work to obtain by any means that could be compassed a strain or strains of the disease in Sydney under our control together with the belief that at that time the incubation period of about four days as given in the text-books was the correct one and that therefore our first injections had failed to produce any result, all contributed to our using so many of the same volunteers for the second injection. Coupled with this was inadvertence on the part of the two of us responsible for the planning out of these experiments in not making it clear that it was advisable to make every possible endeavour to obtain new volunteers to supplement as far as possible previous ones.

The two injections, however, having thus been made with an interval of four days between them, it is necessary to consider what information can be reasonably gathered from the results obtained, either taken alone or taken in conjunction with the other experiments carried out by us. Considering the first injections made on April 8th, Cases 1 to 5 received subcutaneously 1 c.c. Pasteur-Chamberland filtrate from the citrated blood of the Natural Case A taken on the third day of the disease at Murwillumbah. The citrated blood had been outside the body for three days previous to injection. Of these five cases Case 1 received first injection only, and developed no signs of illness whatsoever. Cases 2, 3, 4 and 5 received on April 12th 1 c.c. of citrated blood from the Natural Case C, taken on the eighth day of the disease. Cases 2 and 4 shewed no symptoms of the disease at any time. Case 3 developed an illness of a doubtful nature beginning twenty two days after the first injection and eighteen days after the second injection. Case 4 developed an

apparently definite attack of dengue of a mild type, eleven days after the first injection and seven days after the second injection.

Cases 6 and 7 received as their first injection serum and corpuscles taken on the third day of the disease of Natural Case B occurring at Murwillumbah, the material having been outside the body for four days. Four days later Case 6 received a subcutaneous injection of serum taken on the eighth day of the natural disease from Case C, whilst Case 7 received citrated blood from the same Case C. Cases 6 and 7 developed typical attacks of dengue twelve days after the first injection, and eight days after the second injection.

Cases 8 and 9 received on April 8th subcutaneous injections of clear serum obtained from blood taken on the third day of the natural disease from Case B at Murwillumbah, which material had been outside the body for four days. In addition Case 8 received on April 12th citrated blood taken on the eighth day of the natural disease of Case C, whilst Case 9 received an injection of serum from this same Case C. Case 8 remained well throughout, whilst Case 9 developed a typical attack of dengue eleven days after the first injection, and seven days after the second injection.

A summary of the above results shows that of the five cases injected with the Pasteur-Chamberland filtrate of the citrated blood of natural Case A, three remained well throughout, Case 3 had an illness of a doubtful nature beginning twenty-two and eighteen days respectively after the injections, whilst Case 4 developed dengue eleven and seven days respectively after the injections.

Cases 6 and 7 injected primarily with the serum and corpuscles of Case B both developed typical attacks of dengue eight days afterwards, and four days after the second injection. Of Cases 8 and 9 injected in the first case with the clear serum of Case B, Case 8 remained well throughout and Case 9 developed dengue eleven days afterwards, and seven days after the second injection.

Of Cases 2, 3, 4, 5, 7 and 8 receiving as their second injections citrated blood from Case C taken on the eighth day of the disease, Cases 2, 5 and 8 developed no disease, Case 3 developed a doubtful disease eighteen days after the second injection and twenty-two days after the first, Case 4 developed dengue seven days after the second injection and eleven days after the first, whilst Case 7 developed dengue eight days after the first injection and four days after the second. Of Cases 6 and 9 injected secondarily with the serum of natural Case C taken on the eighth day of the disease, Case 6 developed dengue eight

days after the first injection and four days after this the second injection, whilst Case 9 developed dengue seven days after this second injection and eleven days after the first injection.

It is to be noted that in the only two cases, viz., Cases 6 and 7, receiving injections of the *serum and corpuscles from Case B*, both developed dengue eight days later whilst their second injections consisted in the first case of serum from Case C, and in the second case of citrated blood from Case C. As the only other case (Case 9) which received serum from Case C as a second injection did not develop dengue until the seventh day after this injection, and as of the five other cases which received citrated blood from Case C, as second injections three remained quite well, one (Case 4) developed dengue seven days after this injection, and the remaining one developed an indefinite disease later, it is reasonable to infer that Cases 6 and 7 were infected with the material used in the first injection giving an incubation period of seven days. If this view be correct it cannot be stated whether the second injections also contained infective material, as this would be masked by the positive results from the first injections. The other two cases in this series in which typical dengue developed had one common factor, viz., that they both received injections, in one case (Case 4) of the citrated blood and in the other (Case 9) of serum, from Natural Case C as second injections. The disease appeared in each seven days later. That in this case it is reasonable to attribute the disease to the second injection and not to the first is shewn by the fact that, as regards Case 4, of the four other cases receiving a similar *first* injection three remained perfectly well, whilst the fourth developed an indefinite disease many days later; and that, as regards Case 9, the only other case receiving a similar first injection was Case 8, which remained well throughout. The only other case which received as a second injection serum from Case C was Case 6 in which the infectivity of this material may have been masked by the presumed infectivity of the material first injected.

As our further experimental results shew, in no instance have we found an incubation period as low as four days, or as high as eleven days. On these results the development of dengue in Cases 6 and 7 must be attributed to the first injection, giving an incubation period of eight days, whilst the disease in Cases 4 and 9 must be attributed to the second injection, giving incubation periods of seven days.

(c) *Cases shewing that the virus exists in the blood (serum (and) or corpuscles).*

Owing to the difficulty in preventing blood from clotting, and the necessity of doing a Wassermann reaction before injecting the blood from one person into another, no attempts were made by us to convey blood directly from one individual to another in its natural state. In certain experiments, the serum and corpuscles of blood which had been taken and allowed to clot, were injected subcutaneously, whilst in other cases whole blood was received into citrate normal saline solution, and this, or certain portions of it, were injected.

It is unnecessary to labour the point that the virus exists in some constituent of the blood. The interesting point to ascertain is whether the virus exists in the serum or is in some way attached to the corpuscles. The following cases show that the virus is present in a mixture of serum and corpuscles from clotted infective blood, namely, Case 13, Case 26, Case 27, and Case 29. In Case 28 the result was doubtful, the only indication of a possible mild attack of dengue being a slight rise of temperature for a few days beginning on the fifteenth day. As noted in a special discussion on Cases 2–9 the positive results in Cases 6 and 7 we are inclined to attribute to the injection of mixed serum and corpuscles.

(d) *A case inoculated with whole citrated blood.*

If the positive result in *Case* 4 is to be attributed to the second injection consisting of citrated blood from a natural case of the disease taken on the eighth day of that disease (*vide* discussion on *Cases* 2 *to* 9), as is suggested by the length of the incubation period, then citrated blood as a whole, as might have been expected, is infective, and treatment with citrated normal saline solution is not injurious or at least lethal to the virus.

(e) *Cases in which the Serum of clotted blood was used for injections.*

Of four cases receiving this material, three gave positive results, and one a negative result.

In *Case* 11 in which a positive result followed, the blood was taken on the morning of the third day of the disease and was injected into the volunteer on the evening of the same day. A typical attack of dengue with its rash developed.

In *Case* 25 in which a positive result also followed, the blood had been taken on the second day of the disease and had been kept about eight days in an ice chest before injection. A typical attack of dengue fever resulted.

Case 9, which also developed a typical attack of dengue, received injections of serum, with an interval of four days between them, from two separate sources. *Case* 8 in which a negative result was obtained, received the same first injection of serum as *Case* 9, and four days later a second injection consisting of citrated blood from a different case of the disease. No ill effects followed. From these results in *Case* 8 we attribute the positive result in *Case* 9 to the second injection of serum, the second injection in these cases being the factor in which they differed.

(*f*) *Cases shewing the experimental results with Washed Corpuscles.*

Three cases each received a subcutaneous injection of washed corpuscles from cases of dengue. The corpuscles had been obtained by withdrawing blood from a vein and injecting it immediately into a solution of citrate of soda in normal saline solution. Thereafter the mixture was centrifuged, the supernatant fluid pipetted off and the deposit of corpuscles shaken up with fresh normal saline solution and re-centrifuged. This was repeated from four to seven times. With such material two cases gave negative results, and one a not quite conclusive positive result. In *Case* 10, giving a negative result, the blood had been taken on the third day of the disease, and the corpuscles were injected into the volunteer within twelve hours of removal. Serum from this case derived from the same sample of blood gave rise to a typical attack of dengue fever (Case 11) showing that the blood at this period was infective.

In *Case* 14, which was also negative, the blood was taken on the fourth day and injected into the volunteer the succeeding day. The citrate washings from this case, as detailed later, also gave a negative result in Case 15.

In *Case* 16 an apparently positive result followed. This blood was taken on the second day of the disease and injected into the volunteer on the following day. This illness was a mild one beginning about five days and twenty hours after injection. The patient's appearance and symptoms were those of a mild attack of dengue fever, the temperature reaction was mild, and there was no definite rash. In our opinion the case was a mild one of dengue, though it must be considered as open to considerable criticism.

As inoculation of other material shows that blood still remains infective on the third (and fourth?) days of the disease, the failure of the washed corpuscles to produce the disease in Cases 10 (and 14) and an apparently successful result in Case 16, cannot be considered as dependent on Cases 10 and 14 receiving blood from patients in the third and fourth(?) days of the disease respectively, whilst in Case 16 the patient was only in the second day of the disease. These anomalous results seem rather to indicate that the virus is not of necessity intimately associated with the corpuscles, though it may temporarily adhere to them and be sometimes successfully removed by thorough washing. In other words, these results tend to support the view that the parasite, whatever it is, is not intracorpuscular. If Case 16 be rejected as not being a mild case of the disease, this view is still more strongly supported. On the other hand, if Case 16 be considered a mild case of the disease, the mildness compared with the very definite attack in Case 17, which received the citrated plasma from the same blood, may be considered as shewing that the virus may become attached loosely to the surface of the corpuscles from which by washing it may be to some degree detached, though sufficient virus may still adhere to produce a mild attack of the disease.

(*g*) *Cases in which the fluid part of citrated blood was injected.*

In these cases the blood was received into citrate normal saline solution and then centrifuged. The supernatant fluid was then pipetted off and used for injections. As the centrifuge used was not of very high speed, it cannot be considered certain that the fluid injected was free from corpuscles though these must have been reduced to a minimum. Of two cases receiving these injections, one gave a typical positive result, and one a negative one.

Case 17 (a positive result) followed the use of material obtained on the second day of the disease. Case 16, which received the washed corpuscles of the same blood, developed apparently a mild attack of dengue. Thus the washings of the corpuscles from Case 13 gave a very definite attack of dengue fever in Case 17, as compared with the mild, somewhat doubtful, attack resulting from the washed corpuscles in Case 16. Case 15 gave a negative result. The material in this case was received from Case 11 on the fourth day of the disease. The washed corpuscles from the same blood also gave a negative result in Case 14.

(b) Cases in which a Pasteur-Chamberland Filtrate of the serum and corpuscles obtained from clotted blood was injected.

Of the five cases in which such a filtrate was inoculated subcutaneously, four gave a negative result and one a positive one.

Case 12 which gave a positive result was injected with the filtrate of the clot and serum obtained from blood taken on the second day of an attack of dengue. It was injected the day after collection and was followed by a typical attack of dengue. Unfortunately in this case a test was not made of the reliability of the candle used by inoculating the serum beforehand with a suspension of *B. prodigiosus*. The untreated serum and clot likewise gave a positive result. *Case* 18 which was negative, received the filtrate of the clot and serum from blood taken on the fifth day of a severe case of dengue, the blood having been kept on ice for four days before filtration and the material used on the sixth day from the time of collection. *Case* 19 which was negative received a filtrate of the serum and clot from blood from a case in the fourth day of the disease, the blood being taken one day and the filtrate injected two days later. A local reaction of the arm followed the inoculation, but no attack of dengue. *Case* 20 received a filtrate of the serum and clot from blood taken within forty-eight hours of the onset of an attack of dengue and injected eight days later, having been kept on ice meanwhile. *Case* 21 which was negative, received an injection of the filtrate of the serum and clot from blood taken within forty-eight hours of the onset of an attack of dengue, but which was kept in an ice chest for eight days before injection. In *Cases* 18, 19, 20 and 21 the efficacy of the Pasteur-Chamberland filter was tested by its withholding *B. prodigiosus* added to the serum and clot before filtration.

As regards these negative cases, *Case* 19 received the filtrate from *Case* 11. *Cases* 14 and 15 received respectively washed corpuscles and the citrated washings of these corpuscles from the same case and from the same sample of blood; both of these cases also gave a negative result. This blood was taken on the fourth day of the disease in *Case* 11. These three negative results would seem to indicate that the blood of this case on the date in question was non-infective.

In *Case* 18 the blood was taken on the fifth day of the disease, and there were no other cases inoculated with other samples of this blood to shew whether it was still infective.

In *Case* 20 the blood was taken at a very early period of the disease, —a period during which we know that it is infective. It was kept

outside the body at a low temperature for eight days. In this case the length of time for which the material was kept outside the human body may have tended to destroy the virus, although from *Case* 25 we know that the virus can in some cases at least survive such a period of time. The negative result therefore in this case may be considered to be of some significance.

Similar remarks apply to *Case* 21. In this instance we know that the blood from which the filtrate was taken was infective on the date on which it was removed, as evidenced by the positive results obtained in *Cases* 16 *and* 17.

(*i*) *Cases in which a Pasteur-Chamberland Filtrate of the citrated blood was injected.*

Cases 1 to 5 received injections of a Pasteur-Chamberland filtrate of citrated blood taken on the third day of the disease and kept outside the body for three days before inoculation. In addition to this inoculation, Cases 2 to 5, four days later, each received a second inoculation of material from another dengue case. Cases 1, 2 and 5 remained perfectly well, Case 4 developed a typical attack of dengue eleven days after the first injection and seven days after the second injection. As detailed under "Consideration of Cases 1 to 9," we attributed the successful result in this case to the second inoculation and believe that the filtrate of citrated blood failed to convey infection. In Case 3 an illness developed twenty-two days after the first inoculation and eighteen days after the second inoculation. We are not prepared to say whether this disease was or was not an atypical form of dengue. If the disease was dengue infection could as reasonably be attributed to the second injection as to the first. It therefore appears that none of the five cases injected with the Pasteur-Chamberland filtrate from Natural Case A could with any reasonable certainty be considered as having received the infection from this source. This failure to convey the disease might result either because the blood from Natural Case A was not infective at the time it was withdrawn or that it lost its infectivity during transit and before inoculation, or that the filtration process separated the virus from the filtrate.

(*j*) *Cases shewing the presence of the virus in the blood on certain days of the disease.*

The presence of the virus on the second day of the disease is demonstrated by the results in Cases 12, 13, 16, 17 and 27.

That the virus is present on the third day of the disease is shewn in Cases 11, 25, 26 (32) and 29. If the positive results in Cases 6 and 7 are to be attributed to the first injections (*vide* Discussion on Cases 2 to 9), they also shew that blood is infective on the third day.

If the results in Cases 4 and 9 are to be attributed to the second injection (*vide* Discussion on Cases 2 to 9), then the infective material may still be present on the eighth day of the disease.

(*k*) *Case apparently shewing the absence of the virus after recovery from the disease.*

Case 31 received an injection of serum and corpuscles on the fourteenth day after the beginning of the illness of "B. B.": no symptoms followed. Unfortunately we were unable to inoculate this case later with an active virus so as to shew that he was not naturally immune.

(*l*) *Case shewing the establishment of Immunity shortly after recovery from an attack of Dengue Fever.*

Case 13 received a subcutaneous injection of serum and corpuscles on April 18th, 1916, became suddenly ill on April 25th, and passed through a typical attack of dengue. The temperature reached normal on April 29th but the rash had not completely faded until May 8th.

On June 2nd, 1916, he was reinjected subcutaneously with ·5 c.c. of serum and corpuscles from the case of "B. B." who contracted the disease through mosquito bites. *Case* 13 now became *Case* 30.

On the same date (June 2nd), a non-immune (Case 26 (32) received an exactly similar injection from the case of "B. B." Case 30 remained unaffected as a result of his injection, while Case 26 (32) went through a typical attack of dengue beginning on June 11th. Though it must be borne in mind that it cannot be considered as established that the subcutaneous injection of infective blood from a case of dengue will certainly produce in a non-immune individual an attack of dengue fever, the results obtained in Case 30 point strongly to the view that his failure to develop the disease after an injection of serum and corpuscles known to be infective, was due to his having recently passed through a typical, though artificially produced, attack of the disease. The subcutaneous injection of the new infective material occurred forty-five days from the day when he received his first injection of infective material; thirty-eight days from the onset of his typical attack; thirty-five days from the time when his temperature practically reached normal

after this attack; and twenty-four days from the date on which the rash had disappeared and he was feeling well again—a period at which a definite measure of immunity may reasonably be considered to have been established.

From the results in Case 30 it may therefore be deduced, with the qualification referred to above, that an individual may be completely immune to the virus of dengue fever introduced subcutaneously after a period at least of twenty-four days, say one month, after complete recovery from a typical attack. One cannot say from this experiment that re-infection at an earlier period might not be effective. In other words a definite time may have to elapse before the establishment of any real immunity. We have no precise information on this point. Again how much longer after such an attack this complete immunity remains, is a subject for further investigation. This result is in accordance with the clinical experience of epidemic dengue, namely, that persons who have recovered from an attack are unlikely to suffer from a second attack during the epidemic in spite of the continuance of cases amongst non-immunes several months after these individuals had passed through their attack. There seems, however, some clinical evidence available that occasionally specific individuals may suffer from more than one attack of dengue during the prevalence of an epidemic. There seems little evidence to shew that such immunity exists for periods of time to be estimated in years, inasmuch as victims of one epidemic may be also victims of an epidemic occurring some years afterwards. The rarity, however, of an epidemic occurring in the same area in the immediately succeeding year suggests that some measure of immunity extends over this time. Case 30 would seem therefore to shew, as does clinical experience, that recovery from the disease is associated with an immunity to the disease which probably is the main factor in recovery, and that such recovery is not due of necessity to the organism having completed its life cycle in the human host, though still remaining in this host as a commensal parasite harmless to it but infective to the mosquito. Though it is possible that this may actually be the case, the introduction of further infective material, containing a virus which had not yet completed its possible cycle in the human host, failing in this case to convey the disease, and the clinical experience of immunity to the disease during an epidemic, both point to the view that such an immunity is established, and that it probably plays an important part in recovery from the disease.

(m) Cases showing that the blood can retain its infectivity outside the body for varying periods.

For one to two days. In two of our positive cases (11, 32), the virus was outside the body less than one day. In two of the doubly injected positive cases it was also outside the body less than one day (4, 9). In four cases the virus was outside the body one day (12, 13, 16, 17). In one case two days (29). Thus in nine of our positive cases the virus was outside the body less than two days.

For four days. If the successful results in Cases 6 and 7 are to be attributed to the first injection (*vide* consideration of Cases 2 to 9), then the virus can exist without losing its infective properties for a period of four days outside the body at a mild early autumn temperature such as it was exposed to in transit from Murwillumbah to Sydney. Also in Case 27 the material was kept cool in an ice chest for four days before inoculation.

For seven days. In Case 25 the material was kept in an ice-chest for seven days before inoculation.

From the above we can conclude that the infective agent of dengue fever can survive in the blood outside the body for a period of four days and, sometimes at any rate, longer, viz., up to seven days.

Further work should be done upon this aspect.

(n) Cases showing the length of the incubation period of the inoculated disease.

From 5 to 6 days.

Case 16. 5 days 20 hours.

Case 17. Under 6 days (to onset of fever; 7 days to onset of other symptoms).

Case 29. 4 days 21 hours to first symptoms; 6 days 8 hours to taking to bed.

From 6 to 7 days.

Case 9. 6 days 16 hours (if attack attributed to second injection; 11 days if attributed to first injection).

Case 12. 6 days 14 hours.

Case 13. 6 days 14 hours.

From 7 to 8 days.

Case 27. 7 days 21 hours.

From 8 to 9 days.

Case 6. 8 days 3 hours (if the disease were due to the first inoculation; just over 4 days if due to the second inoculation—*vide* Consideration of Cases 2 to 9).

Case 7. 7 days 20 hours (if the disease were due to the first inoculation; just over 4 days if due to the second inoculation—*vide* Consideration of Cases 2 to 9).

Case 11. 8 days 13 hours.

Case 25. 9 days.

Case 26 (32). 8 days 12 hours.

(*o*) *Is the length of the incubation period dependent on the strain of the virus, or on the susceptibility of the patient or on both?*

We have found by the inoculation of blood and by our mosquito-fed cases that the incubation period of the disease may vary from a little over five days to nine days. The question arises as to why such a variation exists. It is well known in most diseases that such a variation of several days between the shortest known incubation period and the longest known incubation period does exist.

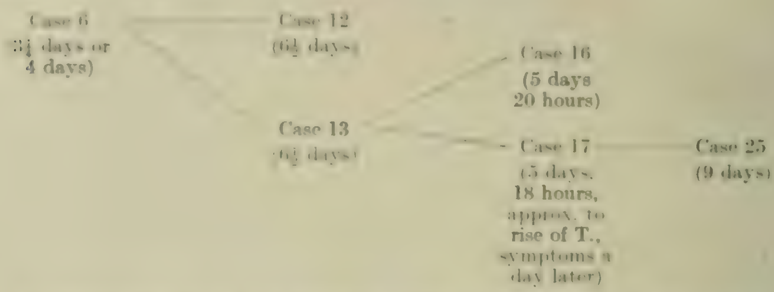
Are such variations due to mutational differences in the virus, to greater or less resistance in the patient, or to differences in the amount of infective materially originally received?

It can be at once understood that a dose of the infecting organism not much above the minimum might result in a prolonged incubation period owing to the longer period perhaps required for the organism to multiply sufficiently to produce symptoms and signs.

Provided, however, that the dose is a reasonably large one, is there any evidence to show that the incubation period will vary with the virus or with the susceptibility of the patient?

The results in Cases 12 and 13 and in Cases 16 and 17 would seem to suggest that the same virus in a sufficient dose tends to produce a disease with approximately the same incubation period. Case 12 received a Pasteur-Chamberland filtrate of clot and serum from blood taken on the second day of an attack of dengue (Case 6), whilst Case 13 received the untreated serum and corpuscles of the same blood. Both were inoculated at the same time, and both developed the disease contemporaneously six and a half days later. In Case 16 the volunteer received an inoculation of washed corpuscles, and in Case 17 the plasma in citrate normal saline solution, the blood in both cases being derived from the same patient (Case 13). The two cases received their inoculations within fifteen minutes of each other, and Case 16 developed dengue fever five days and twenty hours later, whilst Case 17 developed the disease, as indicated by a rise of temperature alone, apparently a few hours earlier (the exact time has not been noted). Case 25 inoculated

from Case 17 had an incubation period of nine days. This sequence of cases can be graphically represented as follows:



An examination of this series of cases seems to shew that the same virus during its passage through a series of individuals may produce illnesses with varying incubation periods of from six and a half, possibly four days, to nine days, but that if the virus be taken at any particular moment and injected into two individuals, it may result in practically identical incubation periods. In other words it would seem that the length of the incubation period is determined more by the state of the virus than by the state of the patient. It should be noted further that the menstruum in which the virus was obtained varied somewhat in the individuals of each pair, so that presumably different doses of the virus were received by the individuals of each pair.

In considering these results, however, due consideration must be given to the fact that only two instances of equal incubation periods are dealt with, and that in Case 17 the temperature rose nearly a day before any symptoms were manifested so that the early incidence of the disease would have been overlooked had the temperature not been taken. The results, however, indicate that further work might very well be carried out to ascertain whether the hypothesis suggested is one of general applicability or not.

(p) *Case Sequences in Relation to Immunity.*

Under this heading are included those instances in which the particular virus has been passed in succession by inoculation from one individual to a second, from the second to a third, and so on. The following is an instance of such successful sub-inoculations.

In Case 6 the virus was present on the second day of the disease as proved by successful sub-inoculations into Cases 12 and 13. From Case 13 further successful sub-inoculations were made from material

taken on the second day of the disease and injected into Cases 16 and 17. From material taken from Case 17 on the third day of the disease a further successful sub-inoculation was made into Case 25. In this particular series we have been successful in conveying the disease by inoculation and sub-inoculation consecutively into four individuals. The virus from which Case 6 was inoculated was presumably obtained from the blood of Case "B" on the third day of the natural disease (possibly from that of Case "C" on the eighth day); thereafter in Cases 13 and 17 the virus was obtained from bloods taken on the second and third days respectively of the inoculated disease, and in Case 25 from blood taken on the third day. We thus see that this virus, by the time it reached Case 25, had produced in human beings the following days of disease, viz., 3 + 2 + 3 + 3, without the virus having passed through any stage of its life history in the intermediate host, the mosquito. When it reached Case 25 it was capable of producing a disease in this patient lasting five days, followed nine days later by a relapse lasting another five days, followed nine days later by a relapse lasting another five days. In other words this virus produced in human beings, without going through any phase in the mosquito, sixteen days of fever followed by a relapse of five days of fever. It may be further noted that the disease in Case 25—the end of the series—was as pronounced as in the first case of the series, shewing that there had been no definite attenuation of the virus. Between the various inoculations, this virus had been outside the human body for four days (presumably) before inoculation into Case 6; for one day between Cases 6 and 13; for one day between Cases 13 and 17; and for eight days between Cases 17 and 25; that is to say, that during the period covered by the sixteen days of fever, the virus itself had been outside the human body for a period of fourteen days. The incubation periods of the disease in the four cases forming the series are respectively eight, six and a half, six and nine, making a total of twenty-nine and a half days. We now get the following totals in connection with this virus when inoculated in series, viz., sixteen days of fever, fourteen days outside the body, and twenty-nine and a half days incubating in the body before manifesting the disease. The total number of days obtained by adding these together is fifty-nine and a half, whereas we find that the virus left the original case on April 4th on the third day of the disease and had completed the primary attack of dengue in Case 25 on May 26th, giving a total of only fifty-five days. The discrepancy is easily explained, inasmuch as incomplete days have been taken as full days in estimating the period outside the body,

the days of the disease, and so on. As a matter of fact, therefore, the figures sixteen, fourteen and twenty-nine and a half, more particularly the two former, are each higher than they should be, probably by a day or a little more. The relative proportions, however, still remain. We thus find that in our series in which the virus was passed from individual to individual over a period of fifty-five days, roughly one-fourth of these days was spent outside the human body, a second quarter was occupied by the virus in producing manifest disease, and in about half of them the virus was incubating in the human body. What information can be gathered from these results? The natural disease produces in its victim an illness definitely lasting from five to seven days followed by convalescence. Does the fever end after the five to seven days of illness because the virus has gone through a phase of development and has now reached a stage ready for transmission to another (invertebrate) host but no longer capable of producing a reaction in the human host; or has the fever subsided because the human host has been able to combat successfully and overcome the virus?

Apart from the question as to whether dengue is usually only infective for the mosquito or by inoculation to other human beings during the first three days of the disease (this point is still undecided), the results obtained in this series would seem to indicate that the latter and not the former view is the correct one. If the virus requires seven days to complete its phase of development in the human body, then sub-inoculations in a series of individuals should fail at the end of an aggregate of seven days fever, whereas in our series we were able to produce sixteen days of fever followed by a relapse. The objection of course may be raised that withdrawal of the blood and keeping it for varying periods of time outside the body, associated with the necessary incubation periods, so interfere with the maturation of the virus in the human body, that a considerably longer period might be required for it thus to mature than would be the case could complete maturation occur in one individual. The balance of evidence, however, seems to be in favour of the view that the patient recovers from his attack of dengue because of his protective reaction against the virus rather than that the virus, having passed through and completed a phase of its existence (viz., that productive of disease in man), still remained in the human host in a stage incapable of infecting human beings with disease (= gamete formation in malarial organisms).

The accompanying Chart I represents graphically the history of this virus. The horizontal lines indicate in days the presence of the

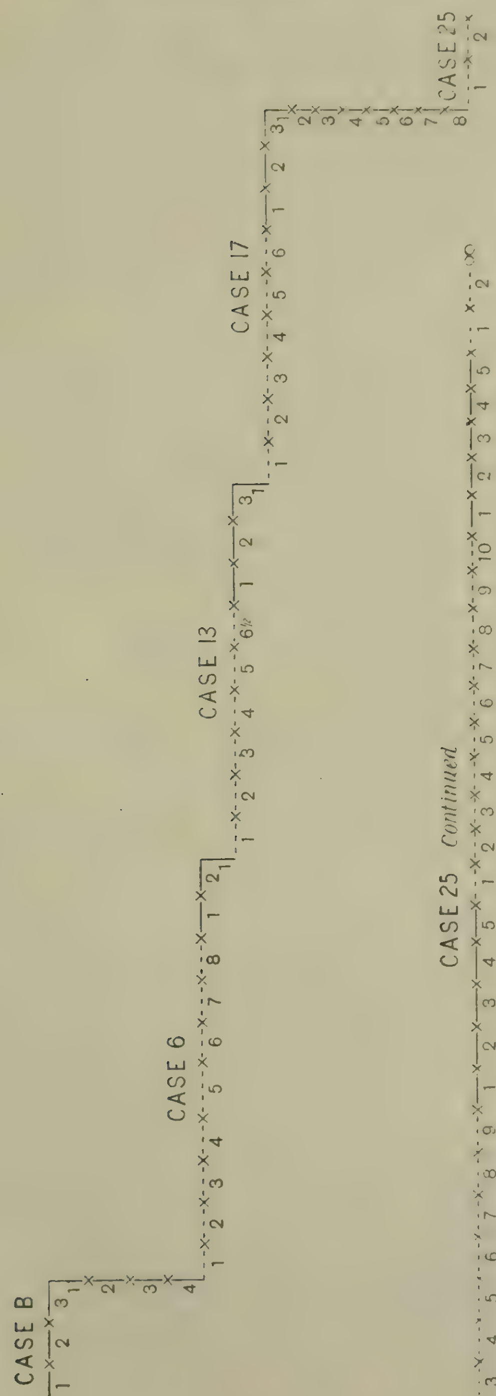


Chart 1. Illustrating case sequences as described in the text.

virus in human beings, whilst the vertical lines indicate similarly the presence of the virus outside the human body *in vitro*. Days marked thus — indicate that on these dates the virus was causing disease in the human victim, whilst days marked thus - - - indicate that it was incubating or had ceased to manifest its presence unmistakeably in the human case.

(*q*) *Can the disease be conveyed by an application of serum to a scarification?*

In Case 22 the arm of the volunteer was scarified as for an ordinary vaccination with calf lymph, and the mixed sera from Cases 16 and 17 were rubbed into the scarified area. The sera were obtained from blood from these cases taken on the third days of the inoculated diseases and at least in Case 17 we know, by the successful inoculation of Case 25, that the virus was present in the material. The result in this case was very doubtful. The patient shewed occasional slight signs and symptoms and a slight rise of temperature, which may have been due to his passing through an exceedingly mild attack of dengue fever. Such a result might perhaps be anticipated if a very mild dose of the virus gained entrance to the human host.

(*r*) *Can the disease be conveyed by an application of infective material to the nostrils?*

In Case 23 the nostrils were swabbed with the mixed sera of Cases 16 and 17 obtained from blood taken on the third days of the inoculated diseases, which we know, in the case of Case 17 at least, to be infective as proved by the successful inoculation of Case 25. The result must be considered as negative, though some very slight symptoms were manifested which may possibly have some significance.

Can the virus be conveyed by the ingestion of infective blood?

As various infectious diseases have been thought at times to be possible of conveyance by the ingestion of infective material, it was thought of interest to see whether gargling the throat and then swallowing a neutral mixture containing infective dengue blood would result in an attack of dengue or not. If in such cases the infection occurred through the pharynx, gargling would facilitate the entrance of the organism, whereas if infection occurred through the stomach or lower down the alimentary canal, swallowing the blood should achieve the

result sought. In Case 24 the mixed sera of Cases 16 and 17, which we know contained the virus, were added to a neutral mixture, and the throat gargled with this, and the material then swallowed. He developed a doubtful but rather suggestive illness, and though his case cannot be considered as being definitely one of dengue, it is nevertheless very suggestive of a mild attack.

In Case 26 blood was similarly used as a gargle and then swallowed, but this blood had been taken from Case 25 on the fourth day of the inoculated disease, and we have no proof by means of other inoculations that it was at this period infective. Case 26 did not develop within the ordinary incubation period any symptoms definitely suggestive of an attack of dengue. In this case the result is somewhat obscured by his receiving subsequently a subcutaneous inoculation of infective material which did produce a typical attack of dengue. It therefore seems clear that at any rate the gargling and the swallowing of the blood did not produce any protective bodies which prevented the patient developing a typical attack by subsequent inoculation of infective material.

(*s*) *The Relationship of Dengue to Yellow Fever.*

The text-books on Tropical Medicine dwell on the differential diagnosis of dengue and yellow fever which may co-exist in places. Neither of us has seen a case of yellow fever and so we are handicapped in considering this aspect of the question. From descriptions, however, it appears that a mild attack of yellow fever may be hard to differentiate from an attack of dengue in the absence of a rash. Castellani and Chalmers state that yellow fever can be differentiated from dengue by its slower pulse, jaundice and haematemesis. From our experience of the clinical disease and of inoculated cases, we have found that a pulse relatively and absolutely slow may occur in cases of dengue, and that therefore this point may not be of material help in a doubtful case. The occurrence of jaundice would be of considerable importance, but we have heard of occasional instances of slight jaundice occurring in dengue patients during the recent epidemic. We have not heard of any cases of haematemesis, but have noted that vomiting has been a sign in some cases of the disease.

We find that the incubation period of the mosquito-conveyed disease and of the inoculated disease in dengue varies from about five to nine days. In yellow fever the incubation period is said to vary from two days twenty-two hours to six days two hours.

We find that the virus of both yellow fever and dengue is transmitted by the same mosquito, *Stegomyia fasciata*.

This shows that there are strong points of resemblance between dengue and yellow fever, and slight, but definite, points of difference. Dengue usually has a definite rash—yellow fever has none. Jaundice and haematemesis are characteristic of yellow fever. The incubation period of dengue is slightly longer than that of yellow fever.

Summary of Results.

1. Dengue Fever in Australia is undoubtedly an introduced disease. It has been existent from time to time in epidemic form since 1885.

2. The clinical description of the disease agrees with that of the Dengue described in text-books, the only departure noted being the distinct tendency to a relatively, and sometimes absolutely, slow pulse rate as compared with the temperature.

3. It is possible that under the single term "Dengue" more than one disease is at present included.

4. Epidemic Dengue in Australia is approximately co-extensive with the known distribution of *Stegomyia fasciata*. It does not extend beyond the area in which this mosquito is prevalent.

5. *Stegomyia fasciata* mosquitoes caught in a dengue infected district in the surroundings of cases of the disease, and some of them known to have fed on a dengue patient on the first and second days of his illness, transported to a non dengue district, reproduced the disease in four out of seven persons on whom biting experiments were conducted.

6. Blood taken from three of these four cases reproduced the disease when injected into further persons. The blood of one case was not tested.

7. The incubation period of the four cases was found to be possibly between five and nine and a half days, probably between six and a half and nine and a half days, counting from the bitings to the definite onsets.

8. No known case of contagion occurred from any of the above four cases.

9. No evidence was obtained from two cases, one of which was heavily and repeatedly bitten with *Culex fatigans*, that *Culex fatigans* is capable of acting as a transmitter of dengue fever.

10. The blood of patients suffering from an attack of dengue can reproduce the disease when inoculated subcutaneously into healthy persons.

11. The disease thus inoculated is typical in every way of dengue fever naturally contracted. The inoculated disease may or may not. shew marked skin rashes and double phases in the temperature charts, and presents a relatively and sometimes absolutely slow pulse; such variations occur in the natural disease. The incubation period of the inoculated disease varies from five to nine days corresponding with the incubation period of the mosquito-transmitted disease.

12. Results of the inoculations shew that:

(*a*) The virus of dengue is present in the blood as a whole.

(*b*) The serum of clotted infective blood may contain the virus.

(*c*) With washed corpuscles one apparently positive result was obtained out of three experiments.

(*d*) The fluid part of citrated infective blood may contain the virus.

(*e*) With Pasteur-Chamberland filtrates of infected serum and corpuscles, one positive result was obtained out of five experiments.

In considering these results failure to convey the disease must not necessarily be interpreted as meaning that the menstruum employed never does contain the virus, as in some of the cases the blood may no longer have been infective at the time at which it was withdrawn.

(*f*) The presence of the virus in the blood has been demonstrated on the second and third days of the disease. Two experiments made may possibly be interpreted as shewing that infective material may still be present on the eighth day of the disease.

(*g*) One experiment appears to indicate that the virus is no longer present in the blood on the fourteenth day from the beginning of the illness.

(*h*) Immunity to the inoculation of infective blood appears to be complete twenty-four days after recovery from a typical attack of dengue.

(*i*) Infected blood may maintain its infectivity outside the body if kept in a cool place for seven days at least.

(*j*) In two instances two individuals inoculated with the same material on the same day exhibited incubation periods practically identical in duration.

(*k*) The infection of dengue can be conveyed by sub-inoculations from individual to individual at least to the fourth generation without the resultant disease departing from the type of the natural disease.

(*l*) The disease has not been conveyed by the application of infective serum to a scarified area; nor apparently has it been conveyed by the application of infective material by swabbing to the nostrils.

(*m*) A very doubtful and probably negative result followed the gargling of the throat with infective material followed by swallowing of the same.

(*n*) Dengue fever has close analogies with yellow fever.

Future Investigations.

The following points require elucidation by further research and we trust that later we may have an opportunity of doing this:

1. To ascertain the period that must elapse after *Stegomyia fasciata* has bitten a dengue patient before the insect can transmit the disease to another human being.

2. To ascertain the length of time that such an infected mosquito may remain infective.

3. To ascertain whether the virus can be transmitted through the eggs to the progeny of such infected mosquitoes.

4. To ascertain whether *Culex fatigans*, *Scutomyia notoscripta*, or any other mosquito can also act as intermediate hosts of the organism of dengue.

5. To ascertain for how long after the third day of the disease the virus may still exist in the blood of the patient.

6. To ascertain how long immunity after an attack may last.

7. A repetition of the experiments suggesting that the virus may be able to pass through a Pasteur Chamberland filter.

8. A repetition of the experiments with washed corpuscles to ascertain whether the organism exists as an intra corpuscular parasite or merely becomes attached to the corpuscles.

9. A repetition of the experiments with serum to ascertain whether the positive results obtained from this source were due to accidental inclusion of infected corpuscles, or liberation of parasites into the serum from injured corpuscles, or whether these results were due to the virus being a natural inhabitant of the serum.

10. A repetition of the experiments with ingested blood and with the application of infected material to the nares and to local scarified areas to ascertain whether the virus so ingested or applied can induce the disease.

APPENDIX I.

DETAILS OF NATURAL CASES OF DENGUE FROM WHOM INOCULATIONS WERE MADE.

Case A. "*Mr P.*" *Murwillumbah.* He first became ill on 3. iv. 16 and was in the midst of a typical attack of dengue with a temperature of 102° F. when blood was taken from him at 11 a.m. on 5. iv. A portion of this blood was injected into citrate normal saline solution, and part was allowed to clot and the serum then separated and sealed. The Wassermann reaction applied to an inactivated portion of the serum proved negative. Owing to contamination the separated serum could not be used for inoculation purposes on arrival in Sydney. A Pasteur-Chamberland filtrate from the citrated blood was used for inoculation purposes in Cases 1 to 5. Cases 1, 2 and 5 remained well. Cases 2 to 5 also received a second inoculation of material from Natural Case C four days after the first injection. Case 3 developed an illness of doubtful nature beginning 22 days after the first injection and 18 days after the second. Case 4 developed an attack of dengue 11 days after the first injection and 7 days after the second. As indicated in our summary of Cases 2 to 9, each of which received two injections, we are inclined to consider that the infection in Case 4 was derived from the second injection and not from the first. This view is chiefly founded on the length of the incubation period.

Case B. "*Mr H.*" *Murwillumbah.* This patient was taken ill on 2. iv. 16. He had pain in the back and down the legs and felt "squeamish" at times. He had had no vomiting. When seen on 4. iv. he was in the midst of a typical attack of dengue with a temperature of 102° F. One eye was congested. His wife at this time was also ill, her attack of dengue having begun on 30. iii. with pain in the back; she also vomited up her dinner. On 31. iii. she felt very sick and had pains in the back and down the backs of the legs, shooting in character, and a sore throat. On 1. iv. she was very sick, and had a rash. When seen on 3. iv. her tongue was clean, with a temperature of 98·4° F. She was covered with a punctate, scarletiniform rash, her hands being also covered with a marked rash. Blood was taken from the husband on 4. iv. and divided into three portions, one being injected into citrate normal saline solution, a second being allowed to clot when the serum was separated and sealed in a tube, whilst the Wassermann test was applied to an inactivated portion of serum with a negative result. The clotted blood from which the serum had been extracted was also kept.

On arrival in Sydney, the citrated blood was found to be contaminated. Cases 6 and 7 received injections from the mixed serum and clot, whilst Cases 8 and 9 received injections of the clear serum. These four cases four days later also received injections of material from Natural Case C. Cases 6 and 7 developed attacks of dengue eight days after the first injection and four days after the second. As indicated under our review of Cases 2 to 9, we attribute infection to the first inoculation, that is, to material from Natural Case B. Case 8 remained perfectly well, but Case 9 developed an attack of dengue eleven days after the first injection and seven days after the second injection. In this case we are inclined to attribute the infection to the second inoculation.

Case C. "*E.S.*" was a patient who had left a dengue district to enter the Coast Hospital, Sydney, to undergo an operation. On arrival at the Coast Hospital he was found to be recovering from an attack of dengue which had been contracted in the endemic area. The history of his movements prior to arrival at the hospital is as follows: He left his home at Tyalgum at 9 a.m. on 31. iii. and arrived at Murwillumbah at 1 p.m. the same day, and had dinner and stayed the night at a boarding-house, leaving by the 6.20 a.m. train for Lismore on 1. iv. He had dinner at Lismore and left for Coraki at 2 p.m. by boat, arriving there at 5 p.m. He stayed at a boarding-house in Coraki from the afternoon of the 1st until the 4th April, when at 7.30 p.m. he left by boat for Sydney. On 4. iv, before leaving Coraki, he had a nasty languid feeling which he could not understand. To get over this feeling he went for a long row in the afternoon, and felt well whilst taking this exercise, but as soon as he got back and became cool again the same languid feeling recurred. He could not account for this feeling until about 9 p.m. the same night on board the steamer at sea, when his eyes began to burn and his bones began to ache. He gradually grew worse until he reached Sydney about 2 p.m. on 6. iv. He stayed at the People's Palace in Sydney until admitted to the Coast Hospital at about 3 p.m. on 10. iv, when he felt in a much improved condition, but was a week in bed at the hospital. He states that whilst at the boarding-house at Murwillumbah he was bitten on the back of the left wrist by a mosquito, and that there was also a dengue patient sleeping in the next room.

On 11. iv. blood was taken from this patient—portion was placed in citrate normal saline solution and portion was allowed to clot and the serum separated. Next day Cases 2, 3, 4, 5, 7 and 8 received injections of the citrated blood, and Cases 6 and 9 of the serum. All these cases had four days previously received injections of material from Natural Cases A or B. Of the cases injected with citrated blood, Case 3 developed a doubtful illness twenty-two days after the first injection and eighteen days after the second injection. Case 4 developed dengue eleven days after the first injection and seven days after the second injection, and Case 7 an attack of dengue eight days after the first injection and four days after the second injection. Cases 2, 5 and 8 all remained negative. As detailed under the consideration of Cases 2 to 9 we attribute the infection of Case 7 to the first injection, and the infection of Case 4 to the second injection, namely, the material from Natural Case C. Cases 6 and 9 injected with the clear serum both developed dengue—the first, eight days after the first injection and four days after the second, and Case 9, eleven days after the first injection and seven days after the second. Here again we attribute the infection of Case 6 to the first injection and Case 9 to the second injection.

Case D. This patient was a soldier who was found suffering from an attack of dengue in camp at Sydney. He had been in camp in a dengue district (Brisbane) for some time. He had spent the previous Saturday, 15. iv. 16, at Sandgate near Brisbane, where he says there were enormous numbers of mosquitoes. He remained well till the evening when he felt "off colour." Next day he had pains in the back and across the loins and had a severe headache especially behind the ears. He says he "saw double." There was eye pain on movement. There was no running at the nose; the throat was dry but not sore. He was ill on Monday, 17. iv, and entrained for Sydney on Tuesday. In the train his temperature was 102° F. During

the train journey he first noticed a rash on the chest on the Wednesday morning. He vomited in the train very severely, the material being pale coloured. He arrived at Sydney on Wednesday night. He was admitted to hospital at 9.30 p.m. the same evening with a temperature on 99·8° F. and a well-marked rash over the chest and back. Next day the temperature was 104°. On Friday, 21. iv, the face was flushed and the eyes injected and he looked ill though he said he was getting better. He still had pains in the back, etc., the eyes were injected and painful, and he was shivering. On enquiry, he stated that he had had dengue twice previously, but not during the present epidemic. On 21. iv. blood was taken from this patient. It was kept on ice till 25. iv. To a portion a Wassermann test was applied with a negative result. The clot and serum was diluted with normal saline solution and passed through a Pasteur-Chamberland filter. Before passing through the filter the material was inoculated with *B. prodigiosus*. Cultures made after filtration proved negative to ordinary bacteria. This material was injected on 28. iv. 16 into Case 18 with a negative result.

Case E. "*J.B.C.*" (*one of us*). He reached the outskirts of the dengue area at Byron Bay at 7 a.m. on 3. iv. 16. At 10.30 a.m. he reached Murwillumbah where the epidemic was still severe, though apparently on the decline. During the rest of this day and on April 4th and 5th he lived in an hotel in the centre of the dengue area, and saw a number of cases of the disease, and caught a number of mosquitoes, both *Stegomyia fasciata* and *Culex fatigans*, in the surroundings of the patients. By means of mosquito netting and citronella oil and other devices, he protected himself as far as possible from being bitten by day- or night-biting mosquitoes. On one or two occasions in patients' rooms *S. fasciata* settled on his arm or face and began inserting their proboscides. These mosquitoes were immediately captured in test tubes. As he is not particularly sensitive to mosquito bites he may have been bitten unawares by other mosquitoes. Though he slept under mosquito curtains at night time, and though he did not find any mosquitoes in the net next morning, he cannot be certain that he was not bitten by such during the night. At about 5.30 on the afternoon of April 5th, he found that the unfed *Stegomyia* in his mosquito cage were escaping through the meshes of the wire, which were hardly close enough to prevent a slender mosquito from wriggling through. As the guinea-pig which had been taken up for the mosquitoes in the cages to feed upon, was injuring the insects and tending to drive the thin ones through the wire meshing, he could not use this animal for distending the bellies of the mosquitoes, and consequently inserted his own hand and forearm to stop the exodus. The *Stegomyia* at once settled upon it and eight or ten at least engorged themselves. These mosquitoes had been collected from houses in which cases of dengue had occurred, and in a number of instances actually from the rooms inhabited by dengue patients. At about 10 p.m. that same night in the dark he inserted his hand into the box containing *C. fatigans*. He left it there motionless for about a quarter of an hour. He did not feel the bites of any mosquitoes, but is relatively insensitive to the bites of this insect, which may therefore have bitten him considerably. On 6. iv. 16 he descended the Tweed River to the Tweed Heads where he saw further cases of dengue and caught more mosquitoes. He reached Brisbane that night, and thinks he may have been bitten by *C. fatigans* but cannot be certain; he left Brisbane at 8 o'clock on the 7th and, with it, the dengue area.

arriving at Sydney at about 11 o'clock on the morning of the 8th with his two cages of mosquitoes.

On arrival, another of us ("B.B."), who had not been to the dengue area at that time, placed his hand in the box containing *Stegomyia*, but for some reason these would not bite him. "J.B.C." then inserted his hand and a *Stegomyia* at once settled upon it and began to pierce the skin. The insect was shaken off and the hand withdrawn.

"J.B.C." remained well until 12. iv. 16 and also on that morning when arising. Later in the morning he felt perhaps very slight and indefinite malaise. A similar condition existed after lunch with a very slight tired feeling. At 5.15 p.m. the tired feeling was more definite, accompanied by the merest trace of headache and a feeling of discomfort in the eyeballs. The tired feeling resembled that of a cold, but with no coryza. His temperature at 6 p.m. was 99·2° F.; at 7.30, 100°; and at 9 p.m. 101·2°, with pulse 102 and respirations 18. He still only felt a tired feeling in the back and the legs with slight giddiness. He spent a very restless night—one of the most restless that he has ever experienced. He kept dropping off to sleep and sleeping for short intervals, and then would awaken suddenly with acute mental alertness. He was unable to get comfortable in any position, and had a slight headache and very slight sore throat, and he sneezed two or three times.

13. iv. At 6 a.m., temperature 98·4° F. A very tired feeling with indefinite pain in the back, legs, and eyeballs. 7.45 a.m., temp. 98·4°. Went into town at 8.15 a.m. At 11 a.m., temp. 100·2°; returned home feeling indefinitely ill; at 2 p.m., temp. 100·8°; slept fairly well in a deck chair from 2 till 4 p.m. At 6.30, temp. 101°, pulse 94; the eyes congested, headache slighter; at 9 p.m., temp. 101·5°; pulse 92.

14. iv. Passed a fairly good night. At 6.45 a.m., temp. 100·2°, slight subcuticular mottling of the abdomen. At 8 a.m. temp. 100°, pulse 84. At noon, temp. 100·2°; at 6.15 p.m., temp. 100·5°, pulse 80; at 9.45 p.m., temp. 100°, pulse 76.

15. iv. Passed a good night. Temp. at 7 a.m., 98·8°; at 3.30 p.m., 99·4°, pulse 80; feeling nearly well; weeded a little in the garden sitting down. At 6.45 p.m., temp. 99·6°, pulse 80, feeling perhaps a trifle more tired than on the previous evening; a bitter taste in the mouth. At 10 p.m., temp. 99·2°.

16. iv. Passed a good night. At 7.30 a.m., temp. 98·6°, feeling stiff; at 2 p.m., temp. 99·2°; at 6 p.m., temp. 100°; at 10 p.m., temp. 99·8°.

17. iv. Temperature at 7 a.m., 99·4°; 1.30 p.m., 100·4°; 6 p.m., 100°; 10 p.m., 100·8°.

18. iv. Temperature at 7 a.m., 99°.

Up to this time "J.B.C." had not "felt himself" since his illness began, but when he awakened on the morning of 19. iv. he experienced his usual feeling of health which was quite different from the feeling on arising the day before, even though there was nothing tangible to be recognised beyond a very slight increase in temperature. Thereafter for several days he felt a certain amount of stiffness of the muscles and of aching during movements of the eyeballs. Also, during his convalescence, he took a dislike to tea and to smoking for a few days. These dislikes fortunately soon disappeared.

Blood was taken from this case on 14. iv. 16 and portion was injected into citrate normal saline solution, and part was allowed to clot and the serum abstracted. On the same day Case 10 was injected with the corpuscles from the citrated blood after

thorough washing, and no illness resulted. Case 11 received an injection of the serum, and eight and a half days later developed a typical attack of dengue with a typical, almost morbilliform, rash. Such a typical rash had been absent during the illness of Case E. Cases 14 and 15 received sub-injections from the successful Case 11 with material taken on the fourth day of the disease and received into citrate normal saline solution. One case received the washed corpuscles, and the other case the citrated plasma, but neither became ill.

APPENDIX II.

DETAILS OF FIRST SERIES OF MOSQUITO EXPERIMENTS.

A. EXPERIMENTS WITH *STEGOMYIA FASCIATA*.

At Murwillumbah on April 3rd, 4th, and 5th a number of *S. fasciata* were caught in the rooms of persons suffering from dengue or on the mosquito curtains of their beds, a few being also captured in houses where cases of dengue had recently occurred. These were supplemented by a few further mosquitoes caught on April 6th at Tweed Heads in the rooms of dengue patients. These mosquitoes were contained in a chocolate box with a wire gauze front and a sleeve of mosquito netting leading to an opening on one side. A small vessel with water was placed at the bottom of the cage. A guinea-pig was taken with us for the purpose of allowing the mosquitoes to feed upon it, but owing to the smallness of the cage and the hairy coat of the animal, attempts to use it for feeding purposes were not successful and caused damage to a number of the mosquitoes, so it was dispensed with. The wire gauze was unfortunately not of a fine enough mesh to prevent a slender *Stegomyia* from occasionally wriggling through. As a number were escaping in this way, on April 5th at Murwillumbah at about 5.30 p.m. one of us (J.B.C.) inserted his arm into the cage when eight or ten out of about forty mosquitoes in the cage settled on his hand. These could be seen distending themselves with blood, and yet no sensation of pain or discomfort was at any time felt. On April 6th at Tweed Heads two dengue patients, one a sailor with a high temperature, and the other a Kanaka, placed their hands in the cage and several mosquitoes at least bit each individual. From Tweed Heads the mosquitoes were taken to Brisbane and thence to Sydney, which was reached on April 8th about 11 a.m. Thereafter the following persons were bitten by these mosquitoes in Sydney:

April 8th, 11 a.m.: One of us (B. B.) placed his hand in the cage; one or two mosquitoes apparently bit him but for some reason they would not feed freely. The other of us (J. B. C.) was accidentally bitten by one mosquito at the same time. The Assistant (W. T.) who accompanied one of us was also bitten by one of the mosquitoes, but this was barely allowed to draw blood. "J. G.," Laboratory Assistant to one of us who had volunteered for these experiments, was bitten by eight mosquitoes at 12.45 p.m. At 7 p.m. one of us (B. B.) was bitten by one of the mosquitoes.

April 9th: About twenty mosquitoes alive. "B. B." bitten by one in the morning.

April 10th: Owing to the shaking of a motor bicycle a number of mosquitoes were on this day unfortunately injured, only three remaining alive. One of these bit "B. B." in the morning. The volunteer "J. G." could not induce any of the three to bite him at 5 p.m.

April 11th: "J. G." bitten by two mosquitoes at 9.15 a.m.

April 12th: "J. G." bitten by two mosquitoes at 9.15 a.m.

April 13th: Mosquitoes would not bite "J. G." Two still alive.

Of the four persons bitten by this batch of mosquitoes, one (J. B. C.) developed a mild attack of dengue on the afternoon of April 12th. It seems probable that the disease was contracted by the mosquitoes which fed upon him at 5.30 p.m. on April 5th at Murwillumbah. "B. B.," "W. T.," and "J. G." developed no symptoms indicative of dengue fever at this time.

B. Experiments with *Culex fatigans*.

A number of *C. fatigans* were collected at Murwillumbah on April 3rd, 4th and 5th and at Tweed Heads on April 6th. These were all caught in rooms inhabited by dengue patients, either on the walls or on the mosquito netting. They were kept in a chocolate box with gauze wire front, a small vessel of water being placed at the bottom during the night time in which the eggs could be laid. At 10 p.m. on April 5th at Murwillumbah, and on April 6th at Brisbane, one of us (J. B. C.) inserted his hand into the cage for about twenty minutes. On neither occasion were any bites felt, but the bite of this mosquito is often not felt by the individual attacked. The mosquitoes reached Sydney on April 8th. They were taken to the Coast Hospital where a volunteer ("— McC.") placed his hand in the box in the dark at 7.30 p.m. Only about eight mosquitoes were alive in the cage and the volunteer thought that four of these bit him. At 9 p.m. one of our Assistants ("J. O. S.") put his hand into the cage and was, he thinks, bitten by two of the mosquitoes.

April 9th: "— McC." inserted his hand again at 7.30 p.m. but felt no definite bites though the mosquitoes settled on his hand. Several bit "J. O. S." at 9 p.m.

April 11th: "— McC." at 7.30 p.m. placed his hand in the box. Apparently none of the mosquitoes bit. "J. O. S." at 9 p.m. was bitten by one mosquito with certainty.

Thereafter neither of these individuals showed any signs indicative of dengue.

APPENDIX III.

DETAILED HISTORIES OF THE FOUR SUCCESSFUL CASES IN WHICH THE VIRUS OF DENGUE WAS CONVEYED BY *STEGOMYIA FASCIATA* IN THE SECOND SERIES OF MOSQUITO EXPERIMENTS.

Case I. *J. G.*, aet. 18, male, laboratory assistant. Not previously in a dengue district. Subject of unsuccessful biting experiments (*Stegomyia*) on 8. iv, 11. iv, and 12. iv.

11. v. 16. Bitten by some twenty eight *Stegomyia* at 2.15 p.m.

19. v. Quite well in the morning. He felt a slight headache first in the afternoon. He came into town at about 7 p.m., to be bitten by mosquitoes, and while sitting with his hand in the cage noticed a feeling of heat and that his headache was worse. He went to lecture after this, and had to go out of the room and go home. He had no evening meal, and went to bed feeling shivery and hot at the same time, and spent a restless night. No vomiting occurred. Incubation period, eight days five hours.

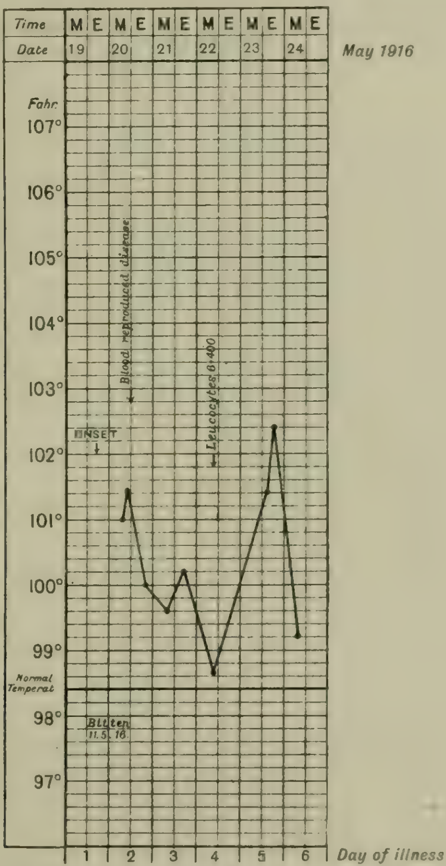


Chart II. Mosquito Case I. J. G.

20. v. He came in to work. One of us (B. B.) saw that he was ill, and that he had a typical dengue face, swollen, hot, and purplish in colour. The headache was severe in the frontal region. His eyes felt as if they were "being pulled," and hurt somewhat to move. There was pain in the neck, and abdominal pains early in the morning. A weak feeling, as in influenza, but no definite pains were complained of elsewhere. There was no coryza, but the eyes were injected. Anorexia was marked, but no nausea complained of. The tongue was furred, creamy at the back, and the

tip typically strawberry. There were three motions since rising. Temperature 101° F. at 9 a.m.; 101·5° at 11 a.m.; pulse-rate 105—see chart. We noted an indefinite subcuticular mottling. Blood was taken for injection experiments. The Wassermann test was negative. The patient was sent home too ill to work.

21. v. Stopped at home.

22. v. He came into town. There was doubtful mottling on the back and forearms. The tongue was still furred, with strawberry tip and edge. His legs were very painful. We took blood for injection experiment. The patient said that he felt "pretty well." Blood examination: leucocytes, 6400; polymorphonuclears, 78 per cent.; mononuclears, 22 per cent. (100 cells counted). No parasites were found in the blood.

23. v. Temperature at 7 a.m. was 102·2° F. He felt worse, the head and eyes were bad. There was an indefinite mottled rash on the chest, arms and back. Temperature at 4 p.m. 101·4°, pulse rate 120. The face was flushed and he looked sick. No obvious coryza was noted.

24. v. The temperature at 7.30 a.m. was 99·2° F. The head was not aching, and the eyes were better. The back was stiff on waking and on bending. He felt fairly well. A definite, slight, mottled rash was seen on the back, lumbar region, and abdomen. It may be described as "midway between measles and scarlet fever, only much less marked."

After this the patient was well, and continued to work as usual.

One c.cm. of the blood was taken from this patient on 20. v, and a volunteer (H. K., Case 27) was injected on 24. v. 16, and became ill eight days later (1. vi, mid-day), and had a typical attack of dengue, with a double temperature curve, typical symptoms and slow pulse.

One c.cm. of the blood was also taken from this patient on 22. v, and was injected into a volunteer (N. McA., Case 28) on 24. v, but no definite attack of dengue developed. This patient's temperature had a definite tendency to be above normal from the start, several times rising to just over 99° F., and on 8. vi, the afternoon temperature was 102°; on 9. vi, at noon, it was 100°, in the afternoon, 102°; on 10. vi, at noon, it was 101°, in the afternoon it was 100·8°; on 11. vi, at noon, it was 99·2°, and in the afternoon 101°. After this the temperature, taken once daily, was normal. He did not complain of any symptoms. The second injection led, therefore, to a doubtful, but probably a negative, result, as the definite febrile reaction that occurred did so fifteen days after the inoculation, suggesting that it arose from some other cause.

Case IV. *Wm.* aet. 27, male, laboratory assistant.

14. v. 16. Between 11.50 a.m. and 12.30 p.m. he was bitten by *Stegomyia*; thirty-six bites were counted.

15. v. At 12.30 p.m., about twenty-two *Stegomyia* bit; at 4.30 p.m., about fourteen *Stegomyia* bit.

Cold in the head during the last few days, but subsiding on 20. v.

20. v. On going to bed he had headache, and passed a bad night. He had a sore throat. The temperature was not taken. The onset occurred at about 9 p.m. The shortest possible incubation period was five days five hours, and the longest possible incubation period was six days nine hours.

21. v. He said that his "eyes, ears and all joints and parts of body are painful." He stopped in bed all day. The headache was frontal, at the back of the head and "behind the eyes." His gums were tender. There was pain in the neck and spine; it was very bad in the lumbar region. "Every part of body was aching." Nausea was present, and he had no appetite; there was no diarrhoea; no delirium was noted;

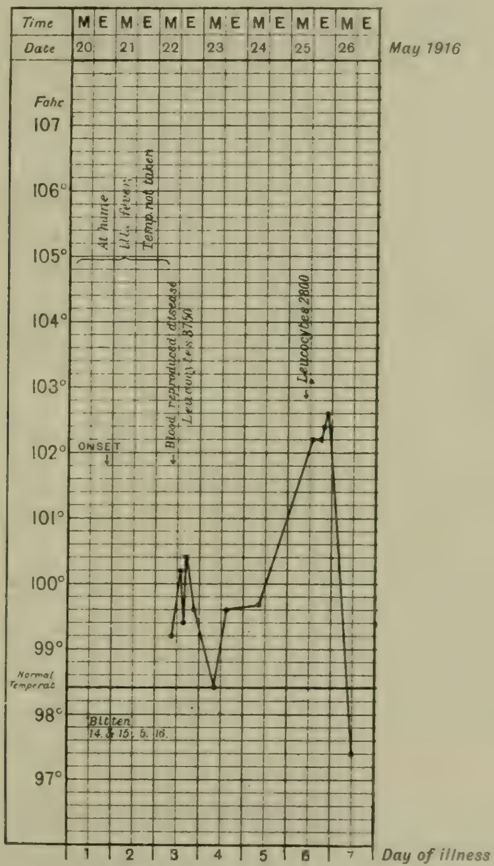


Chart III. Mosquito Case IV. Wm.

he was slightly constipated. He had no cough, but a sore throat. Some coryza was present. The temperature was not taken.

22. v. The patient got up at 8 a.m. Giddiness was present, and shivering. He vomited twice, and was much nauseated, and had no appetite. He came in to be examined. A definite rash, "midway between those of scarlet fever and measles," was found on the arms. It was doubtful on the back. This man said that his rash was often very definite on the arms on waking in the morning, but faded later. He looked ill, and shewed a swollen, typical "dengue face." The tongue was furred in

the centre and slightly strawberry at the tip. He was sent home too ill to work, and was very nauseated in the tram. The temperature on arrival at the laboratory was 99·6°, and the pulse-rate 82. The temperature later in the day was higher (see chart). Blood was taken from a vein for injection experiments. The Wassermann reaction was negative. Blood examination: leucocytes, 3700; polymorphonuclears, 53 per cent.; large mononuclears, 9 per cent.; lymphocytes, 30 per cent.; transitionals, 8 per cent. No parasites found. Red cells normal. (Amalgamated count by J. B. C. and B. B. Only 100 cells counted altogether.)

23. v. On rising, at about 9.30 a.m., the temperature was 98·4° F. On arrival at the laboratory at 10.15 a.m. he complained of an oppressive feeling in the chest and headache. There was a rash on his back, subcuticular, measly, mottled, not well marked. It was still visible on the arms, especially on the under-side of the forearms. At 4 p.m. he went home, as his back and head felt too bad to continue work, and he looked flushed and sick. His temperature was then 99·6° F.

24. v. He did not feel very well on rising, but was fairly well afterwards. His temperature, taken once only, was 99·7° F., and his pulse-rate 96.

25. v. In the morning he felt fairly well, but towards 2 p.m. felt much worse. The headache was severe and there was a tired aching in his limbs. The temperature was 102·2°. Blood examination: leucocytes, 2800; polymorphonuclears, 59 per cent.; lymphocytes, 30 per cent.; mononuclears, 7·5 per cent.; transitionals, 7·5 per cent.; eosinophiles, 1 per cent. (Two hundred cells counted.) Red cells normal. No parasites. The Widal reaction was negative. Rash. This was definite, but slight, on the back, chest and abdomen, and on the anterior internal surface of the right upper arm, over the biceps near the rolled up cuffs, was a collection of slightly raised papules, which disappeared in a day or so. Elsewhere there was a faint measly rash.

26. v. The temperature was subnormal. The man returned to his work nearly well. After this he regarded himself as well.

Blood from this case, taken on 22. v. 16, was injected into volunteer P. S. (Case 29) on 24. v. The latter complained of drowsiness and aching eyes at 1 p.m. on 29. v, but his temperature was subnormal, and he was placed in bed at 6 p.m. on 30. v, when his temperature was found to be 101° F. He went through a typical attack of dengue, with rash and slow pulse, but without the double temperature curve.

Case V. *M.*, 27, female, trained nurse. Previous history: She lived in the North Coast district about eight years ago. About eight or nine years ago she had two attacks of (?) dengue (a year or more apart). She spoke of the sudden onset and extreme pains, but says she did not notice a rash.

16. v. 16. She was bitten by eighteen *Stegomyia* at noon.

25. v. She was well all day, until about 10 p.m. While sitting sewing and listening to music, she suddenly felt sick and tired, with pains in her knees, and went to bed shivering, and did not sleep until 4 a.m. She said she did not feel as if feverish, and did not take her temperature. Incubation period: nine days and ten hours.

26. v. She got up with a headache over the eyes and across the temples. The eyes were painful to move. The morning temperature was 98·6° F. She worked

all day, though not feeling well, having some nausea, but no vomiting. Occasional shivering occurred. The tongue was clean. Her eyes were slightly red and the conjunctivae of the lids swollen; no coryza or cough was noted. She complained of slight sore throat. The fauces were slightly red, but nothing very definite was seen. Temperature: 6.15 p.m., 99·6°; 9 p.m., 100·6°. She stated that she had no rash.

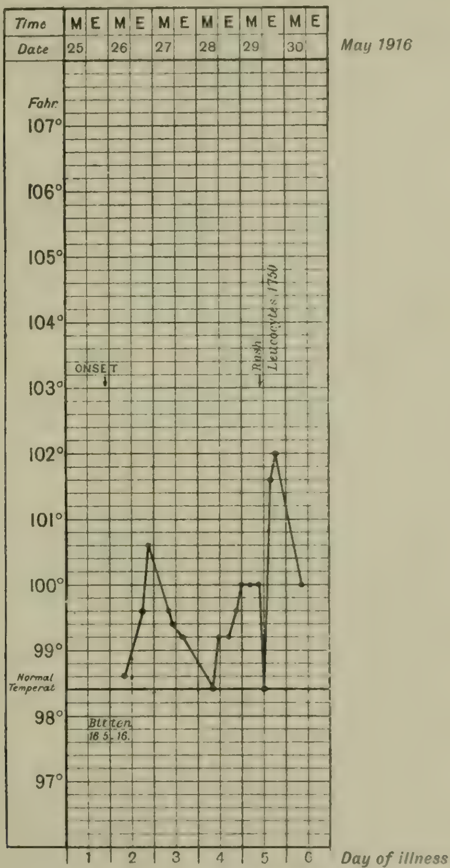


Chart IV. Mosquito Case V. M.

27. v. The temperature at 7 a.m. was 99·6° F.; at 11 a.m. it was 99·4°, and at 4 p.m. 99·2°. The eyes were slightly jaundiced, and the ears slightly yellow, but she said the jaundice was more marked before she got ill on 25. v. 16. The face was flushed, and she said her eyes were painful on movement, but the other symptoms were better. She had no pains in the neck, but had pains across the back and down the back of the legs, and occasionally a feeling of nausea. No rash was noted. She was not examined, except her arms, face, etc.

28. v. She stopped in bed, as she usually did on Sunday morning, for rest. On examination (B. B.) the temperature was normal. The skin was mottled over the back, chest and arms, not a definite rash, but abnormal. The elbows were red and pimply-looking, not very marked. She said she felt weak. Glands were found enlarged in the anterior triangle of the neck, on the left side; but these may have been present before. The temperature went up in the evening (see chart).

29. v. At 9 a.m. she stated that she had had a very bad night, and could not rest at all. She had a recurrence of symptoms, and felt and looked sick. The headache was severe last night, and she took aspirin with relief. On rising, the Matron described a well-marked, measly rash on her arms, which faded on exposure to cold. It was scarcely perceptible at 9 a.m. A mottled, indistinct rash was now on the back. The elbows shewed a very marked and curious condition. The affected areas were about the size of a crown-piece, red, raised, of a bright pink colour, and in the outlying parts were separate papules. She said they were painful to touch. This most distinct condition was seen by the Matron and B. B. Blood examination: no parasites seen. Red cells normal. Leucocytes (duplicate counts made), nineteen whole millimetre fields counted, 1750. The morning temperature was 100·2°; at noon it was normal, and it was up again at night to 102°. The tongue was furred in the centre, and strawberry-like at the tip.

30. v. The patient said she had had a very bad night. Yesterday afternoon she got worse, felt very ill and could not rest at all at night. She could not lie in any position. She said never before had she felt so bad. On examination at 9 a.m., she looked ill; her face was flushed. The tongue was dirty, but not as much as yesterday. The temperature was 99·8°.

The rash was very marked on both arms. It was for the most part of a dark purplish pink colour, and measly in type. It was most marked on the external and extensor surfaces of the fore and upper arms to the shoulders. Over the elbows it was more raised and slightly papular. Very distinct discrete macules were fairly numerous on the palms of the hands. On the upper chest and on the upper back there was an indefinite mottling; on the lower back the mottling was more marked, but not as distinct as on previous occasions. On the knees there was a discrete, small papular rash over the anterior surface for about six to eight inches. On the ankles there was a similar discrete, papular rash on the anterior surface, extending about four inches up the leg and slightly on to the dorsum of the foot.

She was seen by Drs Paton, Armstrong, Van Someren, Woolnough, and by Dr Bligh on the evening before, when the rash was distinct.

She stated to one of us later that, about this time, the rash was marked on the abdomen.

After 30. v. we did not see this patient, and she stopped taking her temperature, but she informs us that she was feeling sick at irregular intervals for a day or two, and suffered for about a week from marked pruritus, especially of the palms of the hands, severe enough to prevent her sleeping.

B. B. saw her next during his own illness, on Sunday, 4. vi. 16, when she was apparently quite well, but still complaining of the itching. Later she said she had occasional headaches for about a week after 30. v.

Case VI. *B. B.*, aet. 34, male, medical practitioner.

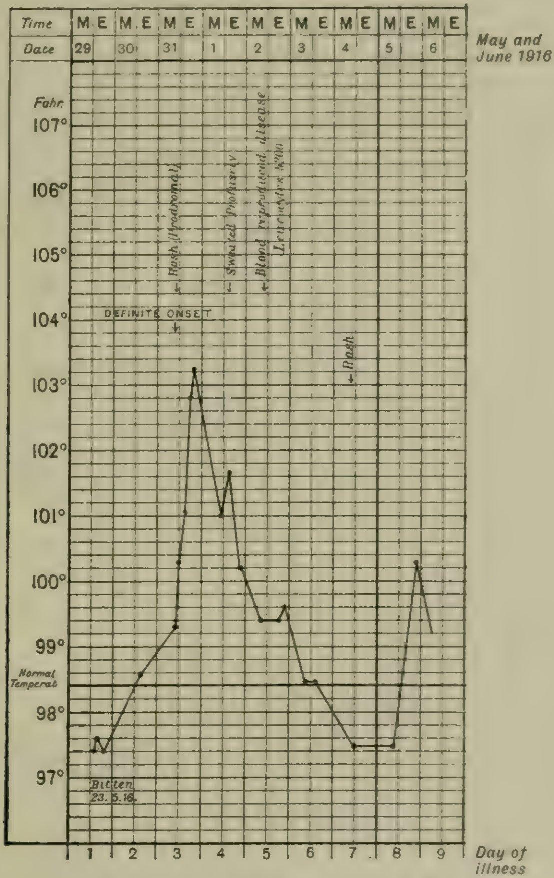


Chart V. Mosquito Case VI. B. B.

13. v. 16. This was the last time B. B. was in the dengue natural area. He was bitten by mixed Grafton mosquitoes.

14. v. He was bitten by mixed Grafton mosquitoes, and arrived back in Sydney (mid-day).

23. v. He was bitten by *Stegomyia*. Fifteen bites were counted. Time, early afternoon.

29. v. He felt quite well on rising, but during the morning, while working, he had shooting pains in the head. In the middle of the day he had definite slight headache and a slight "tired" feeling, and slight pains in the legs and arms. The temperature at 2 p.m. was 97·4° F.; at 4 p.m., 97·6°; and at 7 p.m., 97·4°. The symptoms were so slight that B. B. felt inclined to put them down to imagination, the wish to acquire dengue being father to the thought. He now regards these symptoms as prodromal, and think they might not have been noticed in a non-expectant individual.

30. v. He was feeling "off colour," with occasional slight attacks of nausea, and had a tired sensation in the limbs and slight headache; he was worse towards evening, when he felt cold, shivery and tired, and went to bed early. He passed a rather disturbed night. The temperature at 4 p.m. was 98·4° F.

31. v. On rising, he had malaise, headache, nausea, shivering, pains all over (arms, legs, across shoulders, neck, spine, knees, ankles), and general headache. This increased during the day. The eyes were not very bad, but he was conscious of them feeling abnormal. He had slight sore throat and post-nasopharyngeal irritation, but no coryza. He felt unutterably weary, and could not concentrate his attention. The temperature at 11 a.m. was 99·3° F.; at 1 p.m. it was 100·3°, and the pulse-rate was 80; at 3.45 p.m. the temperature was 101·1°, and the pulse-rate 96. A prodromal rash was present. The incubation period was about seven and three-quarter days to the onset of fever.

Description of prodromal rash seen by Dr Chapple: "A rash resembling subcuticular petechial areas, varying in size; it is most marked on the abdomen, but also present on the back. It is most prevalent in lumbar region posteriorly. The arms are not markedly affected. Each area shews no point of deepest intensity, and the edge is not sharply defined. The colour does not completely disappear on pressure. The colour might be described as a light raw ham colour, with a faint tinge of purple."

J. B. C. Obscure mottling on the trunk; on the back the hair follicles prominent.

B. B. was seen by several medical men. All agree that a distinct rash was present.

Later in the afternoon he felt worse, but managed to work until 4.30, when he went home. He arrived home at 6 p.m. At 6.30 p.m. the temperature was 102·8° F.; at 8 p.m. it was 103·3°, and the pulse-rate was 116.

Note at 8 p.m. The patient was sitting by a gas fire; he felt very hot, but not very ill. Body pains, while resting, were not troublesome. He was very nauseated after tea, of which he ate moderately.

After going to bed, at 9 p.m., he had slight vomiting and marked nausea, and was very restless in bed, the pains in the ankles being maddening. He could only rest by protruding his feet outside the clothes, and could not bear the weight of the clothes. Once he got to sleep he slept well.

1. vi. 16. The patient lay in bed all day. The headache was bad, and was accompanied by photophobia and eye pain. Shivering occurred at times, and giddiness on standing. He sweated twice profusely late in the day. There was slight mental wandering at night, but he slept fairly well.

2. vi. He woke up with headache and eye pains still present, but feeling better, and went into town, though feeling rather shaky, very tired and depressed, nauseated and headachy. The temperature in the morning was 99·0°, leucocytes, 5200. Blood drawn for injection experiments. He went to bed at 7.30 p.m.

A person (G. D., Case 26/32) inoculated with blood taken on 2. vi. 16, developed typical mild dengue. Another person (E. H. R., Case 30), subject of a previous experimental attack, remained well (see injection results).

3. vi. He did nothing all day. Headache was present, and his eyes were tender. He felt very depressed.

4. vi. The patient felt much the same as on the preceding day. The nausea was marked, especially after food. There was a well marked rash all over the trunk. This rash was distinctly different in colour from the prodromal rash. The lesion

might be described as an irregular, fairly bright mottling of the skin; irregular dark areas and irregular pale areas alternated. On the darker areas were brighter punctiform lesions. One of us (B. B.) likens the rash to the strawberry. The rash was best seen early in the morning, and was then noticed on the back of the wrists and on the flanks and back, being less marked on the abdomen. Later in the course of the illness it was well-marked on the forearms. The elbows were somewhat red, but not distinctly so; the flexor and extensor surfaces were both involved; the rash was most apparent on the flexor surfaces of the forearms, and was slight on the external aspect of the buttocks and the anterior aspect of the knees, and doubtful on the ankles and palms. The rash was seen by Drs Cleland, W. G. Armstrong, Paton, Woolnough, Isbister, Chapple and others, who all concur as to its definite characters.

5. vi. He felt worse than on the previous day. He was intensely cold, and shivered immoderately. The rash was well-marked on the arms, wrists and trunk. The temperature was subnormal. Nausea and attacks of great giddiness occurred. In the evening, the temperature rose to 100·3°.

6. vi. He awakened feeling better, and apparently afebrile, and had a good breakfast. He then began work. The temperature at mid-day was 98·1°. The rash was still well-marked; it was seen by Drs Cleland, Paton and Armstrong.

7. vi. He still had headache and slight tenderness on moving his eyes. There was still some nausea and tiredness. The temperature at 11 a.m. was 97·6°.

For the rest of the week he was not feeling "himself," although afebrile. There was a tendency to have headache during part of the day, and pain on moving the eyes and stiffness in the joints, back, etc. A bad taste in the mouth was noted, and inability to enjoy smoking. There was also noted some itchiness of the skin and palms of the hands.

13. v. 16. Blood was drawn for injection experiments.

27. v. 16. The skin was peeling on the legs and hands. The patient had symmetrical, bright-coloured patches of rash on the hips and across the back, which were first noticed about a week before. There were irregular bright red areas alternating with pale skin. The skin was very irritable all over. He still had a stiff, painful feeling on rising in the morning. Otherwise he felt quite well.

The patches of rash on the hips gradually faded, leaving some staining.

INJECTION EXPERIMENTS.

G. D. (Case 26–32) swallowed blood from a previous (blood inoculation) case on 24. v. 16, and complained of pains in the head and dizziness from May 28th (four days later) to May 31st, but had no rise of temperature, the temperature being taken once daily. On 2. vi. 16, he was given 1 c.c. of blood from B. B. subcutaneously. He became ill on 11. vi (eight and a half days later), and his temperature rose on 12. vi, in the evening. He had a definite attack of dengue, with a single temperature curve.

Another experiment was made with the same specimen of blood; 1 c.c. was injected into a volunteer (E. H. R., Case 30), who had passed through a typical attack of experimental dengue, commencing on 25. v. 16, and terminating on 30. v (Case 13). No symptoms followed this second injection within a period of fourteen days.

With a specimen of blood taken on 13. vi. 16 (fourteen days from the onset of B. B.'s illness), a volunteer (G. R., Case 31) was injected with 8 minims. No symptoms or signs of dengue followed during the subsequent nine days.

APPENDIX IV

TABULATED STATEMENT OF THE DETAILS OF THE INOCULATIONS AND ALLIED EXPERIMENTS.

No.	Initials and age	Material used for experiment: Injection 1st	Material used for experiment: Injection 2nd	Day of illness on which material was taken: Injection 1st	Day of illness on which material was taken: Injection 2nd	Date of collection of material: 1st	Date of collection of material: 2nd	Date of injection: Injection 1st	Date of injection: Injection 2nd	Period material was outside body: Injection 1st	Period material was outside body: Injection 2nd	Result	Bordet (Wassermann)	Incubation period
1	J. H., 40	4 A	—	3rd	—	5. iv. 16		8. iv. 16	—	3 days	—	Neg.	—	—
2	G. C., 43	4 A	XX	3rd	8th	5. iv. 16	12. iv. 16	8. iv. 16	12. iv. 16	3 days	½ day	Neg.	—	—
3	R. W., 43	4 A	XX	3rd	8th	5. iv. 16	12. iv. 16	8. iv. 16	12. iv. 16	3 days	½ day	?	—	30. iv. 16 22 or 18 days
4	M. J. W., 46	4 A	XX	3rd	8th	5. iv. 16	12. iv. 16	8. iv. 16	12. iv. 16	3 days	½ day	Pos.	—	19. iv. 16 11 or 7 days
5	J. B., 48	4 A	XX	3rd	8th	5. iv. 16	12. iv. 16	8. iv. 16	12. iv. 16	3 days	½ day	Neg.	—	—
6	W. McG., 48	1 C	XZ	3rd	8th	4. iv. 16	12. iv. 16	8. iv. 16	12. iv. 16	4 days	½ day	Pos.	Neg.	16. iv. 16 8 or 4 days
7	J. C., 49	1 C	XX	3rd	8th	4. iv. 16	12. iv. 16	8. iv. 16	12. iv. 16	4 days	½ day	Pos.	Pos.	16. iv. 16 8 or 4 days
8	J. McA., 53	1 B	XX	3rd	8th	4. iv. 16	10. iv. 16	8. iv. 16	12. iv. 16	4 days	½ day	Neg.	—	—
9	J. E., 56	1 B	XZ	3rd	8th	4. iv. 16	10. iv. 16	8. iv. 16	12. iv. 16	4 days	½ day	Pos.	—	19. iv. 16 11 or 7 days
10	J. D., 64	YA Corpuscles from Case E		3rd		14. iv. 16		14. iv. 16		Less than 12 hrs		Neg.	—	—
11	E. C., 51	YB Serum from Case E		3rd		14. iv. 16		14. iv. 16		Less than 12 hrs		Pos.*	Neg.	23. iv. 16 8½ days
12	G. J., 47	Filtered blood from Case 6		2nd		17. iv. 16		18. iv. 16		1 day		Pos.	Neg.	25. iv. 16 6½ days
13	E. H., 44	Blood, Case 6		2nd		17. iv. 16		18. iv. 16		1 day		Pos.	Neg.	25. iv. 16 6½ days

14	T. H., 59	Corpuscles, Case 11	4th	26. iv. 16	27. iv. 16	1 day	Neg.	—	—
15	E. C., 45	Washings, Case 11	4th	26. iv. 16	27. iv. 16	1 day	Neg.	—	—
16	G. R., 55	Corpuscles, Case 13	2nd	26. iv. 16	27. iv. 16	1 day	Pos.	Neg.	3. v. 16 6 days
17	W. W., 38	Washings, Case 13	2nd	26. iv. 16	27. iv. 16	1 day	Pos.	Neg.	? 3. v. 16 6–7 days
18	J. T., 64	Filtered blood from Case D	6th	21. iv. 16	28. iv. 16	7 days	Neg.	—	—
19	McS., 65	Filtrate from Case 11	4th	26. iv. 16	28. iv. 16	2 days	Neg.	—	—
20	J. P., 56	Filtrate from Case 12	2nd	26. iv. 16	4. v. 16	8 days	Neg.	—	—
21	W. F., 63	Filtrate from Case 13	2nd	26. iv. 16	4. v. 16	8 days	Neg. ?	—	—
22	A. C., 19	*Vaccinated* with serum 16, 17	3rd	5. v. 16	6. v. 16	1 day	Pos. ?	Neg.	—
23	R. K., 48	*Nostrils swabbed* with serum 16, 17	3rd	5. v. 16	6. v. 16	1 day	Neg. ?	Neg.	—
24	W. C., 30	*Swallowed* serum 16, 17	3rd	5. v. 16	6. v. 16	1 day	Pos. ?	—	—
25	L. J., 52	Serum from No. 17	3rd	5. v. 16	12. v. 16	7 days	Pos.	Neg.	21. v. 16 9 days
26	G. D., 67	*Swallowed* serum from 25	? 4th	? 24. v. 16	24. v. 16	? 4 days	Neg. ?	—	—
27	H. K., 50	Blood from J. G., No. 1	2nd	20. v. 16	24. v. 16	4 days	Pos.	—	1. vi. 16 8 days
28	N. M., 63	Blood from J. G., No. 2	4th	22. v. 16	24. v. 16	2 days	Neg. ?	—	—
29	P. S., 46	Blood from Wm.	3rd	22. v. 16	24. v. 16	2 days	Pos.	—	? 30. v. 16 5–6 days
30	E. H., 44	Blood from B. B., No. 1	3rd	2. vi. 16	2. vi. 16	3 hours	Neg.	—	—
31	G. R., 49	Blood from B. B., No. 2	14th	13. vi. 16	13. vi. 16	3 hours	Neg.	—	—
32	G. D., 67	Blood from B. B., No. 1	3rd	2. vi. 16	2. vi. 16	3 hours	Pos.	—	11. vi. 16 8½ days

APPENDIX V.

HISTORIES OF CASES IN WHICH MATERIALS FROM CASES OF DENGUE WERE INJECTED, ETC.

Subcutaneous Injection of Filtrate of Citrated Blood taken on the third day of the Natural Disease (Case A), outside the body three days. *Result*: negative.

Case 1. *J. H.*, m., 48, was injected subcutaneously 8. iv. 16 at 3 p.m., with 1 c.c. of filtrate 4 A (Pasteur-Chamberland filtrate of citrated blood of Natural Case A taken on the third day of the disease on 5. iv. 16). The patient remained well, being under observation for at least twelve days. The result was negative.

Same injection as Case 1, followed four days later by a Subcutaneous Injection of whole Citrated Blood taken on the eighth day of the Natural Disease (Case C), outside the body half-day. *Result*: negative.

Case 2. *G. C.*, *m.*, 43, was injected subcutaneously on 8. iv. 16 at 3 p.m., with 1 c.c. of filtrate 4 A (see above), and on 12. iv. was again injected subcutaneously at 7.30 p.m. with 1 c.c. XX (whole citrated blood from Natural Case C taken on the eighth day of the disease, 12. iv.). This case remained well, being under observation at least eighteen days.

The same injections as Case 2, save that the second one was Intramuscular. *Result*: an illness of a doubtful nature beginning twenty-two days after the first injection and eighteen days after the second

Case 3. *R. W.*, *m.*, 43, was injected subcutaneously with 1 c.c. of filtrate 4 A (see Case 1), on 8. iv. 16, at 3 p.m., and with 1 c.c. XX intramuscularly on 12. iv, at 7.30 p.m. He became suddenly ill eighteen days later on 30. iv. about mid-day, complaining of headache, aching eyes and a feeling of drowsiness. He was placed in hospital and on examination on 1. v. shewed a flushed face with injected eyes, a furred tongue, and an injected pharynx, presenting an appearance suggestive of a mild attack of dengue. His skin was hot and a faint rash seemed beginning to appear on the back. The back shewed an erythematous condition and was very sensitive to pressure. This flushed condition of the back was constant. When questioned regarding any running from the nose, he stated he was suffering in that way but it was not apparent at this or at subsequent daily examinations. The morning temperature was 98·6° F., and the evening one 98·4°.

2. v. 16. The headache was still present and the eyes aching and heavy. There was a pink mottling confined to the back with the congested condition of the back still present. Temperature, morning, subnormal; midday, 99°; evening, subnormal.

3. v. No symptoms. The rash extended round the flanks but was not seen in any other area. Temperature, subnormal.

4. v. No symptoms. The rash was fainter but could be demonstrated on the back and less distinctly on the abdomen. Temperature, subnormal.

6. v. No symptoms. The rash was faintly distinguishable on the back. Temperature, subnormal.

7. v. No symptoms. Still a faint pink mottling on the back. Tongue still coated. Temperature, subnormal.

8. v. No symptoms. The rash seemed to be more demonstrable and to be present on the back, abdomen and lower part of the chest. Urine, 1015, acid, no albumen or sugar. Temperature normal.

9. v. No symptoms. The rash has the same distribution as on the previous day. Temperature subnormal.

10. v. ditto.

12. v. No symptoms. The patient was allowed up, the pink mottling of the back being still present.

(During the period 2. v–12. v. 16 he sweated very freely at night—nurse's report.)

19. v. Re-examined. No obvious rash. Feels well but complains of weakness. Temperature subnormal.

20. v. Discharged from hospital. Temperature subnormal.

2. vi. He became ill again with "headache and aches all over."

3. vi. He was sent into hospital. Evening temperature 101°.

4. vi. On examination his face was found to be flushed; the eyes were injected and watery; there was no running from the nose; the tongue was thickly coated; there was a definite congested condition of the back but no rash. He states he "feels very ill with a terrible headache." Temperature, morning, 101·6°; evening, 101°.

5. vi. Headache still bad; tongue coated; no rash. Temperature, morning, 98·6°; evening, 98·8°.

6. vi. Seems well but still complains of headache; eyes not watery; tongue clean. Temperature, morning, 98·4°; evening, 98·6°.

7. vi. Complained of profuse sweating at night since admission; slight headache and occasional cough; tongue cleaning. Temperature, morning, normal; noon, 99°; evening, 98·4°.

8. vi. Pains in the back (lumbar region); no rash; no sweating. Temperature after this date normal or subnormal.

9. vi. Had a good night; feels well; no rash.

10. vi. Feels well. Urine, 1030, acid, no albumen, reduction of Fehling's solution.

11. vi. Still feeling well. Urine, 1026, no albumen, acid, reduction of Fehling's solution.

12. vi. Well. Urine, 1006, acid, no albumen, no reduction of Fehling's solution.

15. vi. Well. Discharged.

Although the symptoms were suggestive and some rash was present, the length of the incubation period, and absence of fever in the first attack make it seem probable that this case was not one of dengue. In any case it is impossible to include it in our positive results.

The same Injections as Case 3. *Result*: positive, not marked. *Incubation period*: seven days from second injection, or eleven days from first injection.

Case 4. *M. J. W.*, *m.*, 46, was injected subcutaneously with 1 c.c. 4 A (see above) on 8. iv. 16 at 3 p.m., and on 12. iv. at 7.30 p.m. intramuscularly with 1 c.c. of XX (see above).

He stated he had never felt perfectly well since the second injection but became definitely sick on the night of 19. iv. with "a feverish feeling and darting pains like rheumatism in all his joints particularly the knees and shoulders." He also had occipital headache.

20. iv. On examination there was found to be flushing of the face; slight injection of the eyes; no coryza; a coated moist tongue; no rash, but an erythematous condition of the back. He complained of pains all over the body, particularly in the joints. Temperature, morning, 101°; evening, 99·8° F.

21. iv. Feels well; no rash. Temperature, morning, normal; noon, 99·8°; evening, 99·8°.

22. iv. Feels well; no rash. Temperature, morning, 99·4°; evening, 99·4°. (Drop to 97·4° at 4 p.m.)

23. iv. The face seems more flushed. Complains of headache, and pains in the right shoulder. Temperature, morning, subnormal; afternoon, 99·8°.

24. iv. Pains in the legs and shoulders, with headache. Temperature, morning, 99·8°; evening, 100°.

25. iv. Slight headache with pains in the muscles of the lower half of the body; tongue still coated and moist. Temperature, morning, subnormal; evening, 100·2°.

26. iv. "Rheumatic pains in the knees and hips, and in the muscles from the hips down"; complains of sleeping badly. No rash. Temperature, morning, subnormal; evening, 99·6°.

27. iv. Occasional pains in the knees and shoulder joint; no rash. Temperature, morning, subnormal; evening, 99°.

28. iv. Still pains in the knees; feels well. Urine clear, 1018, acid, no albumen or sugar. Temperature, morning, subnormal; evening, 99°.

29. iv. Pains in the elbows and shoulders. Temperature, morning, subnormal; evening, 99°.

30. iv. Pains in the knees; headache. Temperature, morning, subnormal; evening, 99°.

1. v. Pains in the knees and hips. Temperature, morning, subnormal; evening 99°.

2–5. v. Pains in the knees and hips. Temperature, morning, subnormal; evening temperature on 2. v. 16, 99°—after this not above normal.

6–12. v. Feels well. Temperature subnormal. Discharged on latter date.

Urine (undated), 1020, acid, no albumen or sugar.

Remarks.

A review of the chart shews some approximation to the double type. This is undoubtedly an irregular diphasic variation with the high points at noon an April 20th and 8 p.m. on April 25th. There are however several intermissions. Relative bradycardia is very marked after the first day, and periods of absolute bradycardia are frequent. The temperature took a considerable period to settle down as seen by reference to the chart.

This was the only case in which the patient described the pains as being "rheumatic." There was no tenderness in or around any of the joints of which complaint was made, so that acute rheumatism could be excluded.

The case is considered a positive one with the invasion on the night of 19. iv. 16,

thus giving an incubation period of seven days from the second injection or eleven days from the first injection. The case was under observation for thirty-five days from the first injection.

Same Injections as Case 3. *Result*: negative.

Case 5. *J. B.*, *m.*, 48, was injected *subcutaneously* on 8. iv. 16 with 1 c.c. of 4 A (see above), and *intramuscularly* on 12. iv. with 1 c.c. of XX (see above).

This case remained well, being under observation at least twelve days. The result was negative.

Subcutaneous Injection of Serum and Corpuscles, taken on the third day of the Natural Disease (Case B) outside the body four days, followed four days later by a Subcutaneous Injection of Serum, taken on the eighth day of the Natural Disease (Case C), outside the body half-day. *Result*: positive. *Incubation Period*: eight days from the first injection, or four days from the second injection.

Case 6. *Wm. McG.*, *m.*, 48, was injected with 0·5 c.c. of 1 C (serum and corpuscles from Case B) on 8. iv. 16 at 3 p.m. and with 0·5 c.c. of XZ (serum from Case C) at 7.30 p.m. on 12. iv. He became suddenly ill on the night of 16. iv. with frontal headache, a "feeling of cold and hot all over," and a dead aching pain in the legs and lumbar region and an acute sharp pain in the back of the neck.

17. iv. 16. On examination, face flushed, particularly the forehead; eyes slightly injected; injection of pharynx; tongue coated and moist. Appetite good. No rash. Sweated freely during the night; slept well. Temperature at 10.30 a.m., 102·3° F.; noon, 100°; midnight, 102°.

The Pasteur-Chamberland filtrate of clot and serum, and the untreated serum and corpuscles obtained from the blood of this case on this date, conveyed the disease to Cases 12 and 13 respectively, after an incubation period in each case of six and a half days.

18. iv. Slight headache; pains nearly gone; feels fairly well; skin active; pains in the muscles of the arms. *Rash.*—A scarlet flush in the axillary line round the waist and on the buttocks (pressure?). Temperature, 8 a.m., 99°; noon, normal; 8 p.m., 100·6°.

19. iv. Face less flushed; tongue cleaning; feeling fairly well; a faint blotchy erythematous rash on the back and shoulders. Temperature, 8 a.m., 98·6°; noon, 100·2°.

20–21. iv. Feels well; rash the same. Highest temperature, 99·4°.

22. iv. Feels well. In the evening of this day (midnight), the temperature rose to 102·6°.

23. iv. Feels well. Temperature, 8 a.m., 100·6°; 8 p.m., 102·2°.

24. iv. Eyes aching; otherwise well. Temperature, 8 a.m., 99°; 8 p.m., 99·8°.

25–27. iv. Feels well; rash the same. The highest temperature was 99·4°.

28. iv. Feels well; rash the same; allowed up. Urine clear, 1025, acid, no albumen or sugar. Temperature normal.

3. v. Feels fairly well.

4–10. v. Quite well but "weak in the legs."

The urine tested on two other occasions shewed:

A. 1010, acid, no albumen or sugar.

B. 1030, acid, no albumen or sugar.

Remarks.

The incubation period was approximately eight days from the first injection, or approximately four days from the second injection.

The duration of illness was about twelve days. The patient was under observation thirty-two days.

The chart shews definitely a double stage of pyrexia, and is the most typical saddle-back chart in our series of injection cases. In this connection one cannot overlook the double injection and the possibility of the double temperature phase being related to this, but consideration of several other cases, notably Case 25, following a single injection, and consideration of certain of the mosquito cases, does not lend support to such a hypothesis. The saddle-back temperature in this case is a classical feature seen in a moderate number of the natural cases, and probably depends on causes not yet understood. The lowest pulse-rate observed was 54 (3. v. 16). The pulse curve follows fairly closely the first access of pyrexia, although it is relatively somewhat slow. During the second rise of temperature the pulse curve remains on approximately the normal level, indicating a definite relative bradycardia. Absolute bradycardia is not marked in this case, although on one occasion the pulse was 54. Although the temperature was moderately high and the case definitely positive (see subsequent inoculations—Cases 12 and 13), the patient's general condition was very good and he made practically no complaint. In fact he complained more after the disease than during it, stating he had "gone off his legs." The rash was very faint.

> Subcutaneous injection of Serum and Corpuscles, taken on the third day of the Natural Disease (Case B) and outside the body for four days, followed four days later by the Subcutaneous Injection of citrated blood taken on the eighth day of the Natural Disease (Case C). (Outside body half-day.) *Result*: positive. *Incubation period*: eight days from the first injection and four days from the second injection.

Case 7. *J. C., m.*, 49, who was injected subcutaneously with 0·5 c.c. of 1 C untreated serum and corpuscles from Case B) on 8. iv. 16 at 3 p.m., and with XX citrated blood from Case C) on 12. iv. 16 at 7.30 p.m., became suddenly ill on 16. iv. 16 at 11 a.m. with occipital headache and "shivery feelings."

17. iv. 16. On examination: Face flushed; no injection of eyes; no coryza; pharynx injected; tongue moist and coated; headache in all regions. *Rash.*—A pinkish, definitely raised, erythematous rash confined to the inner sides of both thighs. Temperature: noon, 99·4° F.; rose to 101·4° at 4 p.m.; at midnight, 99·6°.

18. iv. Headache; feels better; forehead, face and neck flushed like sunburn. Slight pinkish erythematous patches on the front of the chest; large patches in the axillary line and round the waist; rash copious on the back, over the glutei and on the back of the thighs and on their front and inner aspects; slight on the legs; also present on the palmar aspects of the forearms and slightly on the upper arm. Temperature, 8 a.m., 99°, noon, 98·4°, midnight, 99·8°.

19. iv. Still complains of pain in the back of the neck and headache. A much more prominent, bright pink, definitely raised, erythematous symmetrical rash on the inner and front aspects of the thighs, one similar patch on the back. Highest temperature at 4 p.m., 99°.

20. iv. Feels well. Copious pinkish raised patches on both thighs and both forearms; an erythematous blush on the right arm in the morning, and on the same region in the afternoon a definitely *raised* erythematous rash; the same appearance in the lumbar region; rash very itchy. Highest temperature, midnight, 99·8°; other times normal.

21. iv. Feels well. Rash covering most of the body but particularly on the lumbar and gluteal regions; faint on the forearms and thighs. Highest temperatures, noon, 99°; midnight, 99°.

22. iv. Feels well; rash fading. Temperature normal.

23. iv. Feels well; rash almost gone. Temperature normal.

25. iv. Feels well. Temperature, 4 p.m., 99°; other times normal.

26. iv. Allowed up. Temperature normal on and after this date.

28. iv. Urine clear, 1020, no albumen or sugar.

3. v. Complains of pain in the knees. Fine desquamation present.

8. v. Urine 1020, acid, no albumen or sugar.

Remarks.

This case was under observation for thirty-five days from the first injection. The incubation period was eight days calculated from the first injection, and four days from the second injection.

The chart shews some indication of a double temperature curve, with approximately three days between the two high readings of the temperature chart. Relative bradycardia is present in the pyrexial period, followed by absolute bradycardiac periods. Several times the pulse was 56 and on one occasion 54. Later in the record periods of bradycardia alternate with periods in which the pulse was somewhat quicker than normal. The rash in this case was a fairly distinctive one. The patient complained of great weakness after being allowed up, even up to 12. v. 16 (twenty-six days from the onset).

Subcutaneous Injection of Clear Serum taken on the third day of the Natural Disease (Case B), outside the body four days, followed four days later by a second Subcutaneous Injection of Citrated Blood taken on the eighth day of the Natural Disease (Case C). Outside body half-day. *Result*: negative.

Case 8. *G. McA.*, *m.*, 53, was injected subcutaneously on 8. iv. 16 at 3 p.m., with about ½ c.c. of 1 B (clear serum from Case B), and on 12. iv. at 7.30 p.m. with 1 c.c. of XX (Case C—see above). This man remained perfectly well, being under observation thirty-one days, and the temperature being taken once daily.

Subcutaneous Injection of Clear Serum taken on the third day of the Natural Disease (Case B), outside the body four days, followed four days later by a second Subcutaneous Injection of Serum taken on the eighth day of the Natural Disease (Case C), outside body half-day. *Result*: positive. *Incubation period*: eleven days from the first injection or seven days from the second injection.

Case 9. *J. E.*, *m.*, 56, was injected subcutaneously with approximately ½ c.c. of 1 B (Case B—see above) on 8. iv. 16 at 3 p.m., and on 12. iv. at 7.30 p.m. with about ½ c.c. of XZ (serum from Case C). He became ill about 11 a.m. on 19. iv.

with a sensation of shivering. His temperature at 8 o'clock that evening was 99·4°. He complained of pains between the shoulders and in the nape of the neck, with dull pains in the legs, and dizziness.

20. iv. 16. On examination: The face was flushed; the pharynx injected; the eyes injected. There was a suggestion of an erythema on his back but nothing distinctive. He insisted that his trouble was influenzal and a slight coryza lent additional weight to his auto-diagnosis. He was not sent to bed but his temperature was frequently taken as shewn on the chart. Temperature, 100° F. in the morning.

21. iv. Temperature taken once, 98·8°.

22. iv. Temperature taken once, 98·6°.

23. iv. Temperature, morning, 99°; evening, 99·8°.

24. iv. The patient was on leave and went to the races. He had taken a good deal of drink.

25. iv. Re-examined on this date. His face was decidedly flushed. There was no evidence of coryza. He stated he felt a feeling as if he had drunk a little too much alcohol the day before. He had general pains, headache, and some malaise. His skin was examined, the patient meanwhile protesting that there was no rash on him. The examination, however, revealed a profuse rash. This was a pinkish-coloured erythematous mottling, morbilliform in character, of the whole body, including the soles of the feet, and most copious on the back. On both elbows at the same time, and less distinctly on both knees, were raised pink patches said by an observer to be the colour of "washed eosin stains."

This rash when first seen was considered distinctive enough to warrant having a water-colour record taken but on 26. iv. 16, the earliest time on which this could be done, it had faded so considerably that this procedure was considered useless. Temperature, morning, 100·6°.

26. iv. Temperature, 4 p.m., 100·6°; noon, 98·6°; evening, subnormal.

27–28. iv. Feels well. Rash fading. Urine clear, 1020, acid, no albumen or sugar. Temperature practically normal.

29. iv. Feels well. Rash fading.

1. v. Feels well. Rash fading.

2. v. Rash practically gone.

From 26. iv to 7. v, the temperature at times was slightly above normal.

7. v. Temperature up to 100°. No notes made.

Remarks.

The incubation period is six days sixteen hours, calculated from the second injection, or ten days twenty hours calculated from the first injection.

The chart shews a definite saddle-back temperature with approximately five days between the highest points on the temperature chart. A complete pulse record was not taken.

Two things stand out prominently in this case—the extreme mildness of the symptoms and the distinctive character of the rash. The rash observed was evidently the "secondary rash," and the symptoms thought to be influenzal were the beginning of the disease. This patient made no complaint during convalescence.

Subcutaneous Injection of Washed Corpuscles, taken on the third day of the Natural Disease (Case E), outside the body less than twelve hours. *Result*: negative.

Case 10. *J. D., m.*, 64, was injected on 14. iv. 16 at 8.30 p.m. subcutaneously with 1 c.c. of YA (corpuscles from Natural Case E, taken on the morning of the same day, washed free from serum and citrate and suspended in normal saline). He remained well, being under observation for thirty-three days. His temperature was taken once daily.

Subcutaneous Injection of Serum, taken on the third day of the Natural Disease (Case E), outside the body less than twelve hours. *Result*: positive. *Incubation period*: eight days, thirteen hours. Unsuccessful subinoculations from blood taken on the fourth day.

Case 11. *E. C., m.*, 51, was injected 14. iv. 16 at 8.30 p.m. subcutaneously with about 1 c.c. of YB (serum of Case E), taken on the third day of the natural disease.

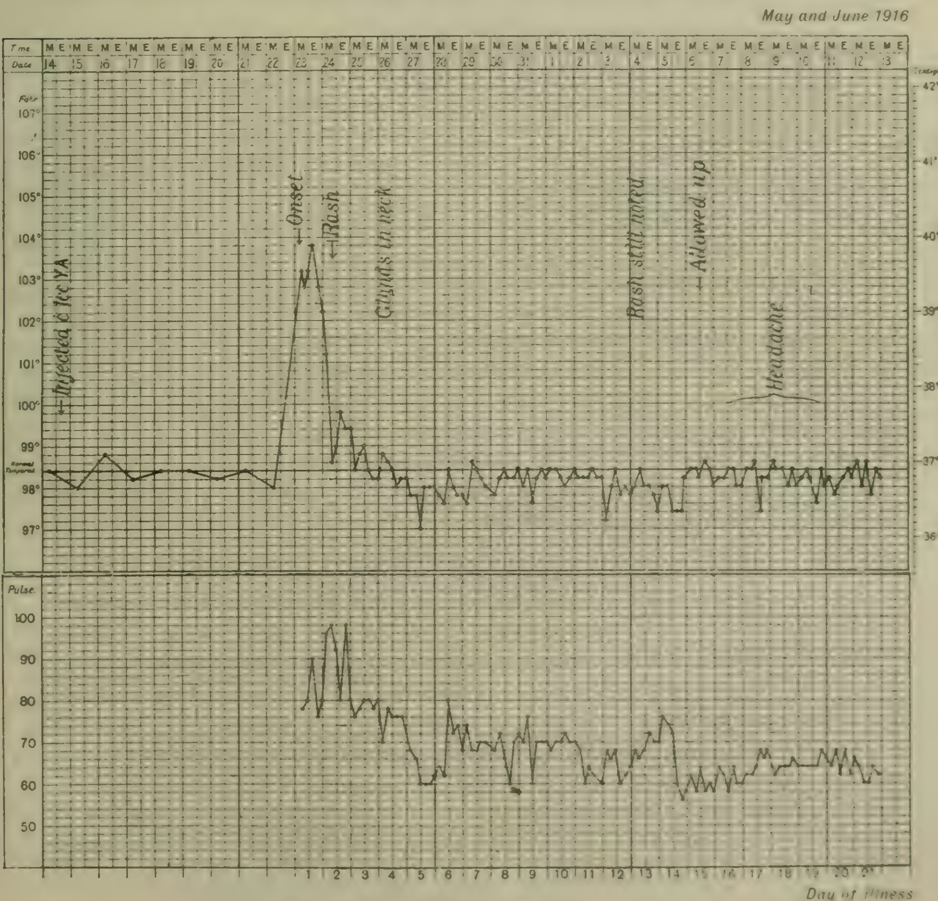


Chart VI. Inoculation Case 11. E C.

23. iv. 16. He became ill on this day with a temperature at 9.30 a.m. of 103·1°. He complained of frontal headache and of a feeling of "being hot all over." On examination: very flushed face; no coryza; no pains; tongue moist and tremulous; pharynx injected; no rash. Temperature, 8 p.m., 103·8° F.; midnight, 102·8°.

24. iv. Headache; well-marked rash over the chest, abdomen, shoulders and thighs. Temperature, 4 a.m., 102·2°; 8 a.m., 102·2°; noon, 98·4°; 8 p.m., 99·8°.

25. iv. Headache improved. Tongue coated and moist; appetite good. A pinkish mottled erythematous rash, morbilliform in appearance, over the whole back; on the front of the chest and abdomen; on the thighs but not on the legs; and on the shoulders, arms and forearms. The rash was mostly in the upper three-fourths of the body. Temperature, 8 a.m., normal; 4 p.m., 99°; 8 p.m., normal.

26. iv. Feels well. Palpable glands in the neck. Rash fading but still prominent on the back. Temperature normal on and after this date.

The subcutaneous injection of washed corpuscles and of the citrated plasma from which the corpuscles were removed, obtained from blood taken on this date, failed to convey the disease to Cases 14 and 15 respectively. The Pasteur-Chamberland filtrate from the blood also failed to convey infection to Case 19.

The blood taken on this date and used for the above injections was treated as follows. Part was citrated and the rest allowed to clot. Some of the clear serum was tested and gave a negative Wassermann reaction. The remaining serum and clot was diluted with an equal amount of a thick emulsion of *Staphylococcus aureus* in normal saline and filtered (Pasteur-Chamberland filter). The water supply failed and in this case the first filtrate coming through was spoiled. The filter was emptied and the unfiltered material collected and refiltered next day (27. iv. 16). Cultures on broth were negative, and subcultures were negative.

The filtrate injected into Case 19 on 28. iv. 16 gave negative results.

The citrate saline mixture was centrifuged and the supernatant fluid removed and replaced with saline. Four centrifugalisations were done and the last being incomplete at 10 p.m., 26. iv. 16, the corpuscles were put into a test-tube with about 20 c.c. saline and allowed to stand in ice overnight, and in the morning were washed again twice, and the corpuscles, suspended in a small amount of saline, retained. Cultures on agar and broth and subcultures from the broth from the preparation on April 26th, and from the final preparation on 27. iv. 16, gave negative results. These corpuscles injected into Case 14 on 27. iv. 16 gave negative results. The supernatant fluid (sterile by broth and agar cultures) after the first and second centrifugalisations was used to inject Case 15 on 27. 4. 16, also with a negative result.

27. iv. Feels well; rash fading.

28. iv. Feels well; rash fading. Urine clear, 1012, acid, no albumen or sugar.

29–30. iv. Feels well; rash fading.

1–2. v. Feels well; rash fading but still noticeable on the abdomen and back.

3. v. Ditto.

4. v. Ditto.

6. v. Allowed up.

7–10. v. Complains of headache. Urine 1020, acid, no albumen or sugar.

Remarks.

The incubation period of this case was eight days thirteen hours. The temperature chart shews a very rapid rise and fall, followed by a more gradual fall to normal, but no saddle-back. The lowest pulse rate was 56. There is well-marked relative and later absolute bradycardia. The slowness of the pulse during the first part of the pyrexia is very marked. The patient complained of headache and general weakness during convalescence. He shewed a well-marked rash, morbilliform in appearance. Subinoculations unsuccessful from washed corpuscles, citrated plasma and Pasteur-Chamberland filtrate obtained from blood taken on the fourth day.

Subcutaneous Injection of Pasteur-Chamberland Filtrate of Clot and Serum, taken on the second day of the Inoculated Disease (Case 6), one day outside the body. *Result*: positive. Unsuccessful subinoculation. *Incubation period*: six days, fourteen hours.

Case 12. *G. J.*, *m.*, 47, was injected subcutaneously on 18. iv. 16 at 7.30 p.m. with 3 c.c. of a Pasteur-Chamberland filtrate of clot and serum from Case 6. This blood was collected, 17. iv, on the second day of Case 6's illness. He became ill suddenly on 25. iv, his temperature at 9.30 a.m. being 102·2° F., and at midday 103·3°. He complained of dull headache in the occipital region and vomiting, but had no pains. On examination: Face very flushed; tongue coated; pharynx injected; slight cough; no coryza; a fine punctate rash on the back with a definite congestion of the skin; skin very hot. Temperature at 8 p.m., 103·2°; midnight, 102·2°. (See Chart VII, overleaf.)

26. iv. 16. Cough without expectoration; eyes injected; bilious vomiting early in the morning. Definite flushing of the back with a fine rash as described. Temperature, 8 a.m., 101·2°; 8 p.m., 102°.

The Pasteur-Chamberland filtrate from blood taken on this date, and injected eight days later, failed to produce the disease in Case 20.

The blood used for the above experiment was treated as follows. Part was allowed to clot and filtration was attempted with a Pasteur-Chamberland filter on the same date after dilution with an equal amount of a thick emulsion of *B. prodigiosus* in normal saline. Owing to poor and intermittent water pressure this was not achieved this day. Later, 27. iv. 16, filtration was effected and cultures from the filtrate on agar and broth and subcultures from the broth remained sterile. The filtrate was used to inject Case 20 on 4. v. 16, with negative results.

27. iv. Cough still present; slight vomiting in the morning; no pain; feels well. The fine punctiform early rash is replaced by a pink, erythematous mottling with definitely raised irregular patches on the back, chest, and abdomen and splashes of erythema on the buttocks (pressure?). Temperature, 8 a.m., 100·2°; midday, 102·2°; 8 p.m., 100·4°.

28. iv. Vomiting; cough with expectoration. Complained of slight headache. Rash the same with some blotchy erythema over the upper portion of the body. Urine clear, 1015, acid, no albumen or sugar. Temperature, 8 a.m., 99°; 8 p.m. 100·4°.

29. iv. Rash still prominent on the back and round the waist. Temperature, 8 a.m., 99·2°; midday, 100·2°; midnight, 99·4°.

30. iv. Feels well; rash still marked. Temperature, normal.

1. v. Rash seems more marked shewing copiously on the whole of the back, the buttocks, and abdomen, and slightly on the chest and upper arms. Temperature normal.

2. v. Face still very flushed; eyes red; complains of slight headache; rash still with the same pinkish mottled character and distribution. Temperature normal.

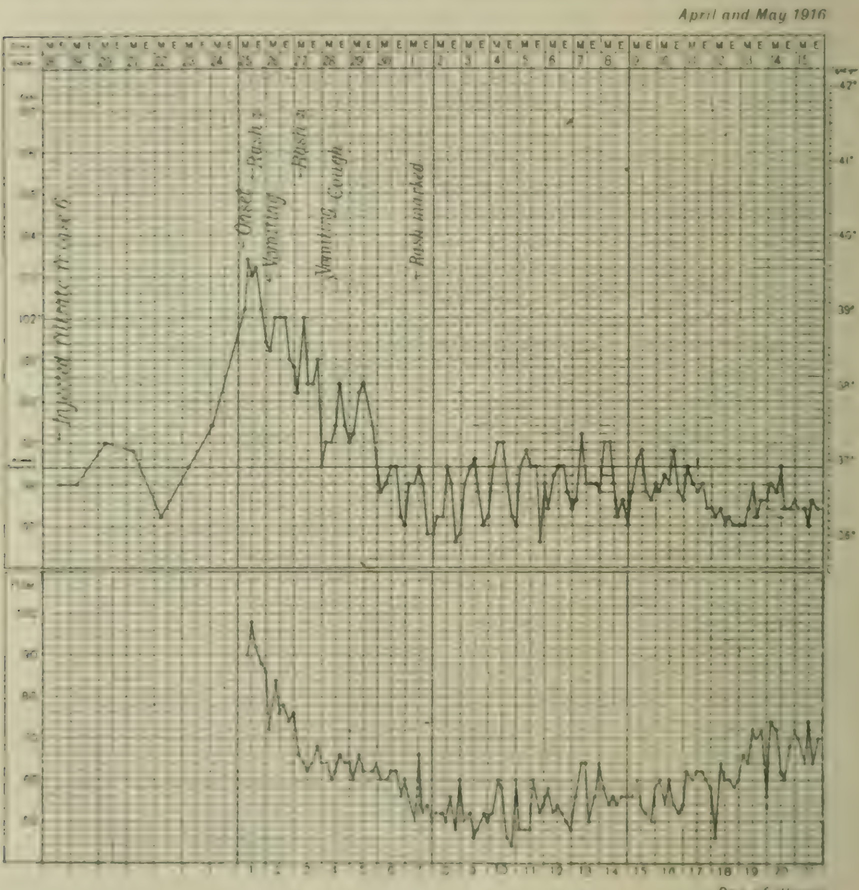


Chart VII. Inoculation Case 12. G. J.

3. v. No symptoms; rash the same. Temperature normal.

4. v. No symptoms; rash fading. Temperature 99° in the middle of the day.

5–6. v. No symptoms; rash fading.

7. v. No symptoms; rash fading.

8–13. v. No symptoms; rash fading. Discharged, 13. v. 16. Temperature, noon, 99·2°.

Urine (undated). 1025, acid, no albumen or sugar.

The case was under observation for twenty-eight days after the injection.

Remarks.

The incubation period was approximately six days, fourteen hours. The chart shews a modified saddle-back although the period of intermission was very short. There is also seen a tendency for the temperature to be above normal from the tenth to the sixteenth day after the onset. The pulse shews throughout the illness marked relative and, after the first week, absolute bradycardia. Indication of the recovery in pulse rate is found during convalescence. The lowest pulse rate was 42. The rashes were fairly prominent features. Vomiting was marked. A negative feature was no complaint of any pains at all in the body or head, other than of slight headache on the fourth day. There was a fine desquamation in this case. This patient also complained for some days of general weakness during convalescence.

Subcutaneous Injection of untreated Serum and Corpuscles, taken on the second day of the Inoculated Disease (Case 6), one day outside the body. *Result*: positive. Successful subinoculations. *Incubation period*: six days, fourteen hours (approx.).

Case 13. *E. H.*, *m.*, 44, was injected subcutaneously with 1 c.c. of untreated serum and corpuscles from Case 6 on 18. iv. 16 at 7.30 p.m. He became suddenly ill on the morning of 25. iv. with headache, which increased in intensity during the day, and aching eyes. The patient stated he was in perfect health on 24. iv. On questioning, his headache was found to be occipital but not severe. On examination: no coryza; face flushed; hyperaemia of the pharynx; tongue furred and moist. Appetite lost.

Rash: a few punctate spots forming a faint rash on the back and between the shoulder blades; back congested. Temperature, 5 p.m., 101·6° F.; 8 p.m., 101°; midnight, 101·2°.

26. iv. 16. Complains of pains in the back all over; slept well. Eyes injected; feels well; glands palpable in the neck. Temperature, 8 a.m., 100°; midday, 102°; 8 p.m., 101°.

The Pasteur-Chamberland filtrate of blood taken on this date and kept outside the body for eight days failed to convey the disease to Case 21. The washed corpuscles after citration and also the washings from these reproduced the disease (Cases 16, 17). (Outside body one day.)

The blood used for the above experiments was treated in a similar way to that from Case 11 (*vide* this case), so that the filtrate, washed corpuscles and washings were obtained.

B. prodigiosus emulsion was used to dilute the serum and clot and filtration was successful on 26. iv. 16. Cultures from the filtrate on agar and broth and subcultures on agar from the broth remained sterile. This filtrate was injected into Case 21 with a (probably) negative result. The washed corpuscles were used to inject Case 16 on 27. iv. 16 with a positive result. Broth and agar cultures made on 26. iv. from these corpuscles were sterile at the time of injection but subsequently a slight growth appeared on the broth culture made from the final preparation. The washings (sterile by broth and agar cultures and broth subcultures), injected on 27. iv. into Case 17, gave a positive result.

27. iv. Complains of pain round the lower ribs and occipital headache. Tongue still coated. A pinkish mottling on the back and the abdomen. Temperature, 4 a.m., 101·2°; 8 a.m., normal; midday, 99°; 8 p.m., 100°.

28. iv. Slept badly during the previous night; complains of pains all over the head and round the lower ribs; feels well. Eyes still injected and tongue coated. The rash is of the same character; it is copious on the back and the abdomen; slight on the chest. Urine clear, 1022, acid, no albumen or sugar. Temperature, 4 a.m., 100°; 8 a.m., normal; midday, 99°; 8 p.m., 100°.

29. iv. Feels well. The rash is copious on the back and the abdomen, and slight on the chest. Temperature, 8 a.m., 98·6°; midday, 98·8°; 8 p.m., 98·6°.

30. iv. Feels well; appetite good. Rash still copious on the abdomen and over the whole back. Temperature normal.

1–2. v. Feels well; rash the same. Temperature normal.

3. v. Feels well; rash fading; eyes red.

4–5. v. Feels well; the rash has faded from the chest but is still present on the back and the abdomen. Temperature normal.

6–7. v. Feels well; rash fading. Temperature normal.

8. v. Feels well; the rash has faded. The patient is allowed up. Temperature normal.

9. v. Temperature, midday, 99°.

Urine (undated), 1018, acid, no albumen or sugar.

Remarks.

This case was under observation for twenty-eight days after the injection. The incubation period was approximately six days, fourteen hours. The patient states he had had "dengue" in Tasmania—a place from which the disease has never been recorded. The chart shews an irregularly remitting febrile period which may be regarded as a much modified saddle-back. There is, as in the last case, a tendency for the temperature to be above normal about a fortnight after the original onset. There is marked relative bradycardia in the latter part of the febrile stage, and occasional periods of slow pulse subsequently. The lowest pulse reading was 52. The rash was fairly copious. The patient made little complaint except during one night, that of 27. iv. 16. Washed corpuscles and also washings from corpuscles reproduced the disease in two cases. A filtrate kept eight days proved ineffective.

Subcutaneous Injection of Washed Corpuscles, taken on the fourth day of the Inoculated Disease (Case 11), outside the body one day. *Result*: negative.

Case 14. *T. H.*, *m.*, 59, was injected subcutaneously on 27. iv. 16 at 3.45 p.m., with 1·5 c.c. of washed corpuscles (blood received into citrate normal saline solution and corpuscles washed several times in normal saline solution) taken on the fourth day of the inoculated disease (Case 11). He remained well, being under observation twenty-nine days. The temperature was taken once daily and was never over 98·4° F.

Subcutaneous Injection of washings of Citrated Plasma freed from corpuscles, taken on the fourth day of the Inoculated Disease (Case 11), outside the body one day. *Result*: negative.

Case 15. *E. C.*, *m.*, 45, was injected subcutaneously on 27. iv. 16 at 4 p.m. with 2 c.c. of the "washings" (plasma in citrate normal saline solution after removal of the corpuscles by centrifuging) taken on the fourth day of the inoculated disease (Case 11). He remained well, being under observation twenty-four days. His temperature was taken once daily; the first day after injection it was 99° F., but thereafter it was never over 98·4°.

Subcutaneous Injection of Washed Corpuscles, taken on the second day of the inoculated disease (Case 13), outside the body one day. *Result*: positive (mild). Inoculation into this, a third individual, successful, but illness mild (perhaps due to a minimum of infective material). *Incubation period*: five days, twenty hours.

Case 16. *G. R.*, *m.*, 55, was injected subcutaneously on 27. iv. 16 at 4.10 p.m. with 0·6 c.c. of washed corpuscles (*vide* Case 14), taken on the second day of the sub-inoculated disease (Case 13). He became suddenly ill whilst having dinner at 12.30 p.m. on 3. v. 16, with pain in the nape of the neck, headache and dizziness. His temperature that afternoon was 99° F. so he was sent into hospital for observation. On examination—his face was flushed; the tongue coated; the pharynx injected; there was slight cough; no coryza; no injection of the eyes; there was congestion of the skin of the back but no rash; there were erythematous blushes round the waist, on the thighs, and on the abdomen (pressure?).

4. v. 16. Pain in the back of the neck; no rash. Maximum temperature 98·6°, 4 p.m.

5. v. Pain in the head and neck; no rash. At midday his temperature shot suddenly up to 101°, but at 4 p.m. it was normal.

Serum obtained from blood taken on this date, mixed with similar serum from Case 17, both being kept two days outside the body, was applied by scarification of the arm, by swabbing the nostrils, and by gargling and swallowing to Cases 22, 23 and 24 respectively, with negative or very doubtful results.

6–8. v. Feels well; appetite good; slept well. No rash. Temperature normal.

9–11. v. Complained of diarrhoea and pains in the lumbar region and down the legs. No rash. On 10. v. 16 at midday the temperature was 99·2°; on 11. v. 16, the temperature at 8 a.m. was 99·6°, and at midday 99·4°.

12. v. Vomited during the preceding night. Temperature, 8 a.m. and midday, 99·6°.

13–14. v. Feels well; no diarrhoea; no rash. After this date the temperature was practically normal.

17–22. v. Feels well.

Urine (undated), 1020, acid, no albumen or sugar.

Remarks.

The incubation period was five days twenty hours. This case is considered as a mild positive one. This view is taken from the patient's appearance, symptoms, incubation period and pulse rate. The temperature chart is not typical but shews a double

phase, the two high points being separated by about five days. The pulse rate was frequently low, 56 being the lowest reading, but the pulse chart is very irregular. Additional weight is lent to the view that the case is positive by the fact that some observers considered that he had a faint rash. As however there was nothing conclusive about the rash, it was not stated in the history. Material from this case was used in three special non-inoculation experiments. Three doubtful results. (See Cases 22, 23, 24.)

Subcutaneous Injection of Citrated Plasma, taken on the second day of the Inoculated Disease (Case 13), outside the body one day. *Result*: positive. Inoculation into this, a third individual, successful. *Incubation period*: six to seven days.

Case 17. *W. J. W.*, *m.*, 38, was injected subcutaneously on 27. iv. 16 at 4.25 p.m. with 2 c.c. of the "washings" (plasma in citrate normal saline solution after the removal of the corpuscles by centrifuging obtained from blood) taken on the second day of the sub-inoculated disease (Case 13).

3. v. 16. His temperature rose on the morning of this date to 99·6° F., and had increased to 100·6° at 5 o'clock in the afternoon. He was thereupon sent to hospital, although he protested that he had no symptoms and in fact never felt better in his life. On examination his face was flushed; the tongue not coated; the eyes somewhat injected; there was no coryza; the appetite was good. No rash.

4. v. He states that the right side of his head is aching badly and complains of pain in both heels and sleeplessness. Eyes injected; tongue coated; no rash; appetite good. Temperature, 8 a.m., 100·2°; noon, 101·2°; 8 p.m., 102·2°.

5. v. Feels weak but otherwise well. An erythematous blush on the forehead and right shoulder and in front of both knees. Temperature, 8 a.m., 100·2°; noon, 99°; 8 p.m., 101°.

Serum obtained from blood taken on this date and kept in the ice-chest for eight days, conveyed the disease by inoculation to Case 25. Serum obtained from blood taken on this day and mixed with similar serum from Case 16, was applied by scarification of the arm, by swabbing the nostrils, and by gargling and swallowing to Cases 22, 23 and 24 respectively, with negative or very doubtful results.

6. v. Complains of stiffness in the muscles but nothing else. No rash. Temperature, 8 a.m., 100·8°; noon, 100·8°; 4 p.m., 102°; 8 p.m., 102·2°; midnight, 102·8°.

7. v. Slight cough but no sputum; tongue coated. A raised pinkish mottling on both shoulders, on the back and in the region of the great trochanters, copious on the abdomen. Temperature, 4 a.m., 100°; 8 a.m., 100°; noon, 100°; 8 p.m., 101°.

8. v. Heavy sweating during the night. Eyes still injected; a reddish coated tongue. The rash was of the same character as previously, being prominent on the back and buttocks, copious on the abdomen, slight on the chest, shoulders and arms, and fairly copious round the lower ribs. Temperature, 8 a.m., 97·4°; noon, 99·4°; 8 p.m. 101·6°.

9. v. Sweating during the night. Rash fading.

10–11. v. Night sweating. Rash practically gone—most prominent round the lower ribs. Temperature subnormal on and after this date (taken till 16. v. 16).

12. v. Feels well; slight nocturnal sweating.

13. v. Feels well but weak; no sweating.

Remarks.

The incubation period was somewhat under six days to the onset of fever, seven days to the definite onset of symptoms. The temperature chart shews an irregular type of chart; the pyrexial period lasted a week and was interrupted by two definite intermissions. It may be regarded as an irregular saddle-back chart complicated by a secondary remission in the second pyrexial period due to the marked sweating which occurred. The pulse is for the most part relatively slow, and there are definite irregularly occurring periods of absolute bradycardia in the post-febrile phase. The recovery of the pulse in the later stages of convalescence is well shewn. The lowest pulse rate was 50. Sweating, as above mentioned, was a pronounced symptom in this case. It is perhaps worth noting that his temperature rose definitely a day before the onset of symptoms. Sub-inoculation of serum was successful.

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood, taken on the sixth day of the Natural Disease (Case D), kept on ice four days, filtered, outside body seven days. *Result*: negative.

Case 18. *J. T., m.*, 64, was injected subcutaneously on 28. iv. 16 at 7.45 p.m., with 2 c.c. of the Pasteur-Chamberland filtrate from the diluted blood of Case D. This blood was taken on the sixth day of a severe attack of dengue and was kept on ice for four days, filtered and was outside the body seven days from the time of inoculation. This man remained well. His temperature was taken once daily for twenty-seven days and was only normal or subnormal, except once when it was 98·8° F. (twentieth day).

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood, taken on the fourth day of the Inoculated Disease (Case 11), two days outside the body. *Result*: negative.

Case 19. *E. McS.*, was injected at 8 p.m. on 28. iv. 16 with 2 c.c. of a Pasteur-Chamberland filtrate of the blood from Case 11, taken on the fourth day of the disease and kept two days outside the body.

29. iv. 16. His arm was swollen and painful, and the temperature at 8 p.m. was 101° F.; midnight, 100·4°.

30. iv. Arm still swollen and sore. Temperature, 4 a.m., 99·8°; 8 a.m., subnormal; noon, 98·6°; 8 p.m., 99°.

1. v. Arm better; some areola. Temperature, 4 a.m., 99·2°; 8 a.m., subnormal.

2. v. Arm is well. Temperature normal.

The patient's temperature was taken four hourly until 10. v. and once daily until 23. v. 16, without shewing any increase above the normal, and no symptoms occurred.

The temperature of 101° F. on the morning following the injection was almost certainly due to some toxic body in the injected material, probably unconnected with dengue.

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood taken on the second day of the Inoculated Disease (Case 12), outside the body eight days. *Result*: Negative.

Case 20. *J. P., m.*, 36, was injected subcutaneously on 4. v. 16 at 6.10 p.m., with 2 c.c. of the Pasteur-Chamberland filtrate of Case 12, the blood being taken on the second day of the inoculated disease (26. iv. 16) and kept in the ice-chest for eight days. He remained well. On 6. v. 16 his temperature was recorded as 99·6°, but taken once daily for fifteen days after this it did not rise above normal.

Subcutaneous Injection of Pasteur-Chamberland Filtrate from blood taken on the second day of the Inoculated Disease (Case 13), outside the body eight days. *Result*: negative.

Case 21. *W. F., m.*, 63, was injected subcutaneously on 4. v. 16 at 6.30 p.m., with 2 c.c. of the Pasteur-Chamberland filtrate from blood taken on the second day of the inoculated disease (Case 13) and kept in the ice-chest for eight days. His arm became swollen and sore the next morning, and his temperature went up reaching a maximum at 5 o'clock in the afternoon of 100° F. This pyrexia was attributed to the presence of toxic bodies in the filtrate, probably independent of the virus of dengue.

The temperature was further taken until 3. vi. 16 (thirty days), and during that period he shewed occasional periods of pyrexia, viz. on 18. v. 16 to 99.3°, and on 27. v. 16 to 100·8°. These were considered as being independent of the injection of the filtrate.

Scarification of the Arm, as for ordinary vaccination, and application of mixed Serums from Cases 16 and 17, taken on the third day of the Inoculated Disease, two days outside the body. *Result*: negative (?). *Incubation period* (if any): seven days.

Case 22. *A. C., m.*, 19, was vaccinated as with calf lymph on the arm with the mixed serums of Cases 16 and 17, taken on the third day of the inoculated diseases, two days outside the body, on 6. v. 16, and was sent into hospital for observation on 13. v. His maximum temperature on this date was 98·8° F.

14. v. 16. Complains of headache; otherwise no other symptoms or signs. Temperature subnormal.

15. v. Feels well; no rash. Temperature subnormal.

16. v. Face a little flushed; eyes slightly injected; no rash but a congestion erythema of the back and erythematous flushes on the buttocks (pressure). Temperature at noon rose to 99·4°; 4 p.m., subnormal.

17. v. Tongue clean. A doubtful faint mottling on the back and thighs. The temperature at 4 p.m. rose to 99°; afterwards it was subnormal.

18. v. Diarrhoea; tongue slightly coated; suggestion of a mottled rash on the back, chest and abdomen. Temperature normal or subnormal on this date and subsequently.

19. v. Feels well; no rash.

20–23. v. Feels well; no rash.

24. v. Feels well, except for aching eyes.

25. v. Allowed up.

Urine (undated), 1020, alkaline, no albumen or sugar.

Remarks.

The incubation period, if any, would be seven days. The highest temperature shewn was 99·4° F. and the lowest pulse rate 52. There seems to have been some reaction in this experiment but so slight that the case must be accepted with due reserve. The vaccination gave no local reaction.

Nostrils swabbed with the mixed Serums of Cases 16 and 17, taken on the third days of the Inoculated Diseases, two days outside the body. *Result*: negative.

Case 23. *R. K.*, *m.*, 48. The mixed serums from Cases 16 and 17 obtained from blood taken on the third days of the inoculated diseases, kept in the ice-chest two days, were applied by swabbing to each nostril on 6. v. 16. The patient was sent into hospital for observation on 13. v. 16.

14. v. 16. No symptoms complained of. On examination, there were no signs. The maximum temperature was 98·8° F.

15–16. v. No symptoms complained of. On examination, there were no signs. The maximum temperature was 99°.

16. v. Urine clear, 1015, acid, no albumen or sugar.

17. v. Feels well; tongue slightly coated; skin reaction to pressure very definite on the back, buttocks and shoulders. Suggestion of a pinkish mottling on the back, chest and abdomen. Temperature normal.

18. v. Face slightly flushed; eyes injected; tongue coated; rash (?), same distribution. Temperature normal on and after this date.

19. v. Feels well; rash (?) the same.

20. v. Feels well; rash (?) faint on the back and chest.

21. v. Feels well; rash (?) faint on the back and chest.

22. v. Feels well. Allowed up. Urine 1025, acid, no albumen or sugar.

Remarks.

There was no certainty as to the presence of a rash in this case, and the general reaction, if any, was so slight that the case must be considered as negative.

The Throat gargled with a mixture in milk, tinted with liquorice, of the mixed Sera of Cases 16 and 17, obtained from blood taken on the third days of the Inoculated Diseases, two days outside the body, the mixture being then swallowed. *Result*: positive (??). *Incubation period* (if any): seven days.

Case 24. *m.*, 30. On 6. v. 16 gargled his throat with and then swallowed a mixture in milk tinted with liquorice of the mixed sera of Cases 16 and 17, obtained from blood taken on the third days of the inoculated diseases, and kept for two days in the ice-chest.

13. v. 16. Owing to the presence of a fine punctate rash on the back, chest and both arms, he was sent into hospital. He complained of no symptoms. Temperature at noon, 98·6° F.

14. v. Complains of headache; rash fainter; no flushing of the face. Temperature all day normal or subnormal.

15. v. No symptoms; tongue coated; eyes slightly injected. Temperature at 4 p.m., 98·8°.

16. v. Temperature at noon rose to 100·4°, but normal before and after this.

17. v. Pains in the legs. A morbilliform rash on the chest, the abdomen and back; raised pinkish patches on the buttocks and thighs; pink erythematous slightly raised patches on the elbows; tongue coated and furred. Temperature normal or subnormal.

18. v. Pains in the right arm; strange in manner (the patient is epileptic); face slightly flushed; morbilliform rash fading. Temperature, 99° at 8 a.m., otherwise normal.

19. v. The morbilliform rash gone; pinkish definitely raised patches on the buttocks and the back; nil on the chest, abdomen, legs and arms.

20. v. Rash on back fading.

21. v. Rash faded; feels well.

Remarks.

The incubation period, if any, would be about seven days. This case may possibly be considered as a mildly positive one. There were practically no symptoms but the character of the rash, the bradycardia (lowest pulse reading 52), and the presence of slight fever (100·4° F. on 16. v. 16) support this view.

The patient insisted on his discharge on 22. v. 16 so we were unable to ascertain by inoculation whether any immunity had been established. An analysis of the urine was not obtained.

Subcutaneous Injection of Serum, obtained from blood taken on the third day of the Inoculated Disease (Case 17), seven days outside the body. *Result*: positive. Inoculation into this, a fourth individual, successful. Relapse. *Incubation period*: nine days.

Case 25. *L. J., m.*, 52, was injected at 7.45 p.m. on 12. v. 16 with (amount not stated) of the serum obtained from blood taken on the third day of the inoculated disease (Case 17), kept in the ice-chest for seven days. He became suddenly ill about 7 p.m. on 21. v. 16 with headache, dizziness and pains in the legs. The temperature at midday was 97·6° F.; the evening temperature was not taken.

22. v. 16. Face flushed; tongue clear; eyes slightly injected; no coryza; no rash. Temperature, noon, 102°; 4 p.m., 103°; 8 p.m., 103°; midnight, 103°.

23. v. Sleeplessness; no headache. A faint punctate rash on the back and abdomen. Temperature, 4 a.m., 102·2°; 8 a.m., 101°; noon, 100·2°; 8 p.m., 98·6°.

24. v. Pains in the lumbar region; eyes injected; sleeplessness. A faint rash on the back. Temperature, 4 a.m., 99·6°; 8 a.m., 99·6°; 8 p.m., 100°.

Blood, used as a gargle and then swallowed, taken on this date failed to convey the disease to Case 26.

25. v. Feels well but suffering from sleeplessness. A faint pinkish mottling in the lumbar region and on the thighs. Temperature, 8 a.m., 98·4°; 8 p.m., 99°.

26. v. Rash. Pinkish mottled patches on the chest and abdomen, particularly in the lumbar area and on the sides of the chest; more definitely raised pinkish masses on both thighs (symmetrically placed). Temperature, 8 a.m., 98·4°; 8 p.m., 98·6°.

27. v. Slept better. Rash the same. Temperature normal from this date until 5 . vi . 16.

28. v. Rash disappearing; slightly present still on the back and legs.

29. v to 2. vi. Feels well. Rash disappearing.

3. vi. Rash gone; allowed up.

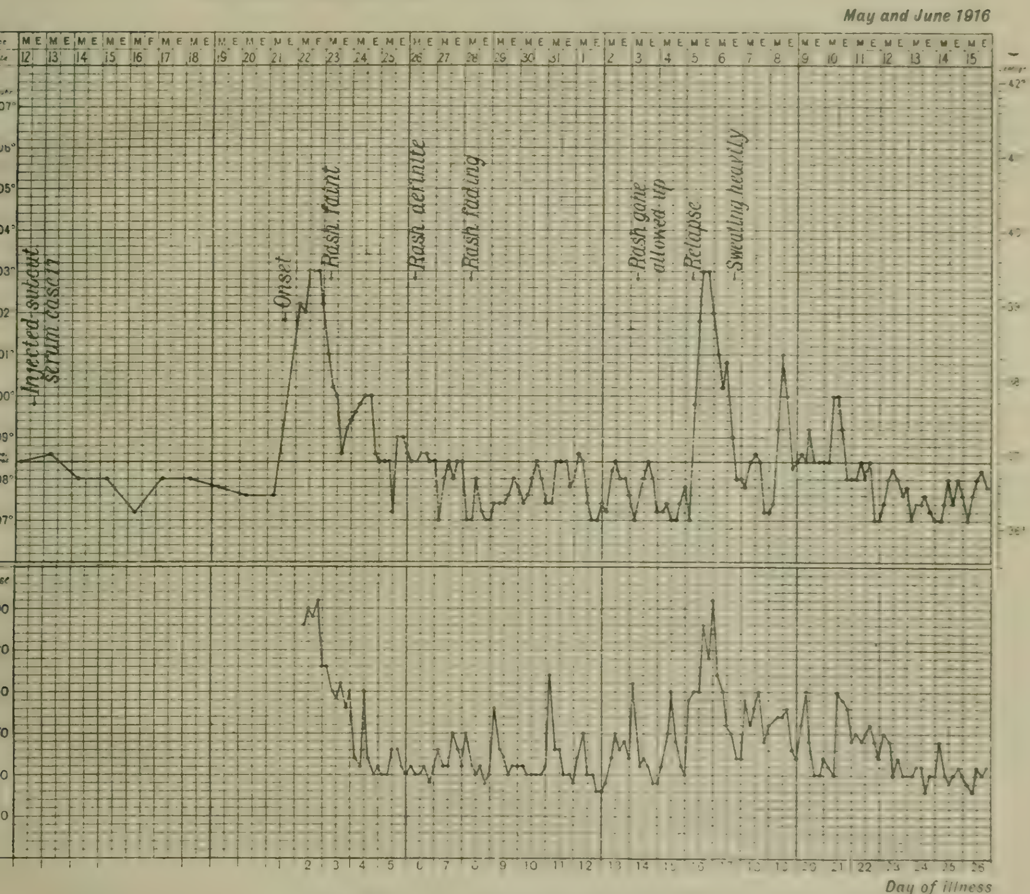


Chart VIII. Inoculation Case 25. L. J.

Relapse? The patient had been walking about for a few days, feeling well, when he became suddenly ill with very severe headache and a high temperature. At noon on 5. vi. 16 his temperature rose to 99·8° F., at 4 p.m. it was 101·8°, and at 8 p.m. and midnight 103°.

6. v. On examination: face flushed; eyes watery. Temperature, 4 a.m., 102°; 8 a.m., 101°; noon, 100·2°; 4 p.m., 100·8°; 8 p.m., 99°; midnight, 98°.

7. vi. Slight headache; slept well; copious night sweats (patient says three or four pints). Temperature normal or subnormal.

8. vi. Feels well; no sweating; no rash. Temperature at noon, 99·2°; 4 p.m., 101°; falling at 8 p.m. to 100°; normal at midnight.

9. vi. Feels well. Temperature reached 99·2° at 4 p.m. (maximum).

10. vi. Slight cough. Temperature reached 100° at midday and at 4 p.m. was 99°; at 8 p.m. and thereafter during convalescence normal or subnormal.

11. vi. Slight sweat and cough. Urine 1016, alkaline, no albumen or sugar.

13–15. vi. Feels well; slight cough.

16. vi. Feels well.

21. vi. Urine clear, 1015, neutral, no albumen or sugar.

The urine tested on two other previous occasions during the course of this case shewed: (A) 1010, acid, no albumen or sugar. (B) 1016, alkaline, no albumen or sugar.

Remarks.

The incubation period of the first attack is nine days, and a relapse occurred fifteen days later. The chart shews a single temperature curve during the first attack, and a typical double curve, with a period of apyrexia of two days, during the relapse. The pulse shews considerable relative and occasional absolute bradycardia. We have described this case as consisting of a single pyrexial period followed fifteen days later by a relapse, but one might consider the two phases as an example of an exaggeration of the two-phase characteristic with a very prolonged intermission.

Blood taken on the fourth day of the Inoculated Disease (Case 25), used as a Gargle and then Swallowed. *Result*: negative. Subsequently, Subcutaneous Inoculation of Serum and Corpuscles, obtained on the third day of the Mosquito-conveyed Disease (Mosquito Case No. 5). *Result*: positive. *Incubation period*: eight days, twelve hours.

Case 26 (32). *G. D.*, *m.*, 67, on 24. v. 16 gargled his throat with and then swallowed a mixture composed of milk tinted with liquorice and blood taken on the fourth day of the inoculated disease (Case 25), used immediately. At 8 p.m. on 2. vi. 16 he was injected with ·5 c.c. of serum and corpuscles taken on the third day of the mosquito-conveyed disease ("B. B." Mosquito Case No. 5), outside the body several hours. He remained perfectly well and free from any symptoms until 2. vi. 16.

2. vi. 16. Headache; coated tongue; appetite good; face flushed; congestion erythema on the back. Maximum temperature, 98·8°.

3. vi. Feels well. Maximum temperature, 98·8°.

4. vi. Flushed face; pharynx injected; eyes watery; feels well; no rash. Temperature normal.

5. vi. Feels well. Maximum temperature, 99°.

6. vi. Maximum temperature, 99°.

7. vi. Some pains in the arms. Temperature normal.

8. vi. Feels well. Temperature normal.

9. vi. Feels well. Urine 1030, acid, no albumen, Fehling's solution reduced.

10. vi. Feels well. Temperature normal.

11. vi. He became definitely ill at 8 a.m. with headache and aching eyes; the tongue was coated; the eyes injected; the face flushed; there was no rash. Urine 1025, acid, no albumen, Fehling's solution reduced. Temperature normal.

12. vi. Headache and pains all over the body especially in the arms and shoulders. In the afternoon he complained of pains in the knees and more intense headache and aching eyes. Temperature, 8 a.m., 98·6°; 12 noon, 100°; 2 p.m., 102·6°; 4 p.m., 102·4°; midnight, 99·6°.

13. vi. Headache; faint mottling on the chest and abdomen. Temperature, 4 a.m., 100·2°; 8 a.m., subnormal; noon, 99·4°; 8 p.m., 99°.

14. vi. Faint mottling on the chest, back and abdomen. A heavy night sweat. Temperature normal after this date.

15. vi. Faint rash on the back, chest and abdomen.

16. vi. Feels well. Rash the same as on the previous day; itchy.

17–19. vi. Rash still the same—pinkish irregular mottled patches on the chest, abdomen and upper arms, doubtfully raised.

20. vi. Feels well. Rash the same. Urine 1017, acid, no albumen or sugar.

21–23. vi. Feels well. Rash on the back.

23–26. vi. Feels well. Rash on the back.

Remarks.

The incubation period is eight days twelve hours from the date of the injection to the onset of symptoms, and a little over nine days till the first rise of temperature. The reaction to the swallowing was very slight, if any, and must be considered as a negative result.

The reaction to the injection was very definite and was certainly positive (compare appearance, symptoms, rash and temperature).

An interesting feature was that the patient complained of typical symptoms twenty-eight hours prior to any elevation of the temperature.

The temperature chart shews a single-phase febrile paroxysm. There is marked relative bradycardia and the pulse rate tends to be slow throughout. It will be noticed, however, and we shall have more to say in this connection in discussing the pulse in these cases, that there is quite definitely a tendency to slow pulse well before the beginning of the attack.

The result of analysing the urine in this case was very surprising—the specific gravity on 9. vi. 16 being 1030, and a reduction with Fehling's solution taking place. Tested on 11. vi. similar results were obtained, but on 20. vi. the specific gravity was 1017 and there was no reduction of Fehling's solution.

Subcutaneous Inoculation of Blood taken on the second day of the Mosquito-conveyed Disease (Mosquito Case No. I), outside the body four days. *Result*: positive. *Incubation period*: seven days twenty-one hours.

Case 27. *H. K.*, *m.*, 50, was injected subcutaneously on 24. v. 16 at 3 p.m., with 0·5 c.c. of the blood of J. G. (Mosquito Case No. I), taken on the second day of illness (20. v. 16) and kept in an ice-chest for four days.

This patient became suddenly ill after dinner (midday) on 1. vi. 16, suffering with headache and aching limbs. Temperature, morning, 98·4° F.; afternoon, 100·2°.

2 and 3. vi. 16. On examination: flushed face; coated tongue; watery eyes; no coryza; loss of appetite; sleeps well; feels "shivery all over." No rash. Temperature on morning of 2. vi. 16, 98·4°; 8 p.m., 99·6°; midnight, 100°. Temperature, 3. vi. 16, 8 a.m., 100°; noon, 100·6°; 4 p.m., 102·8°; 8 p.m., 100·6°; midnight, 102°.

4. vi. Headache severe; feels "shivery"; tongue coated; flushing of the skin of the back; no rash. Temperature, 8 a.m., 100°; 4 p.m., 102·8°; 8 p.m., 101°; midnight, 102·2°.

5. vi. Headache; aching in shoulder muscles; eyes still watery; tongue clearing; no rash. Urine clear, 1018, acid, no albumen or sugar. Temperature, 4 a.m., 99°; 8 a.m., subnormal; noon, normal; 4 p.m., 99°; midnight, 100·2°.

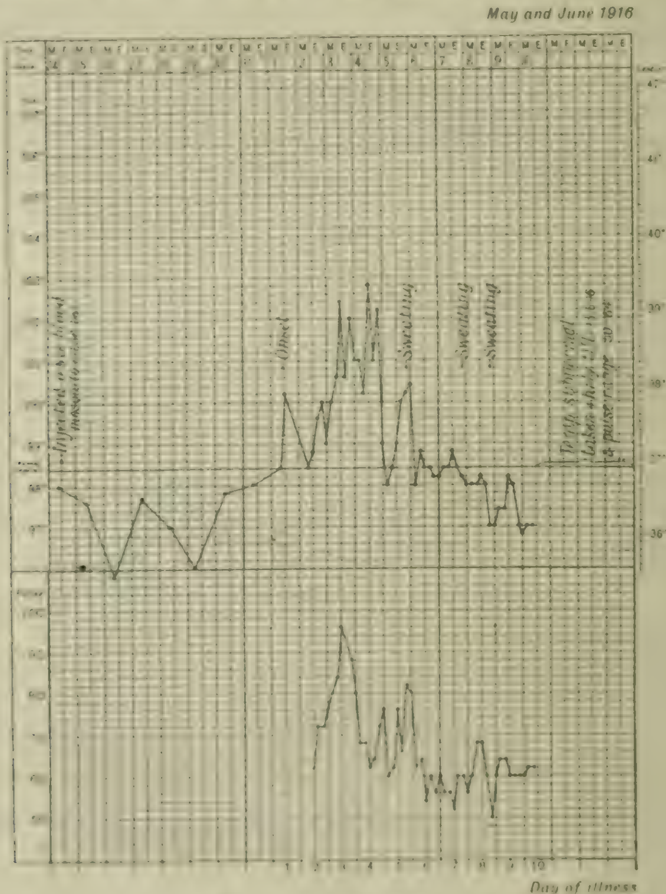


Chart IX. Inoculation Case 27. H. K.

6. vi. Headache; aching in the shoulder muscles; heavy sweating the previous night. Temperature, 4 a.m., 100·4°; 8 a.m., subnormal; midday, 98·8°; afternoon, normal.

7. vi. Feels warm; eyes watery; tongue clearing. Highest temperature at 4 p.m., 98·8°; temperature normal after this.

8. vi. Heavy sweat during the previous night; feels weak; eyes watery.

9. vi. Heavy sweat during the previous night; dizziness; slept well.

10–13. vi. Feels well; eyes still watery; appetite poor; no rash.
14. vi. Aches in the back of the head.
15. vi. Discharged.
19. vi. Urine clear, 1020, acid, no albumen or sugar.

Remarks.

The incubation period is nearly eight days (seven days twenty-one hours). The temperature chart shews an irregular chart with two remissions before the final fall. The pulse shews definite relative and, later on, some absolute bradycardia. The lowest pulse recorded was 50. There was no rash. During convalescence the patient complained of marked general weakness.

Subcutaneous Inoculation of Blood, taken on the fourth day of the Mosquito-conveyed Disease (Mosquito Case No. I), outside the body two days. *Result*: negative (?).

Case 28. *N. M.*, 63, was injected subcutaneously on 24. v. 16 with 1 c.c. of the blood of J. G. (Mosquito Case No. I), taken on the fourth day of illness (22. v. 16). His temperature was taken once daily—on the fourth, eighth, ninth, eleventh and twelfth days it is recorded as being 99° F. On the fifteenth day the temperature was 99·2° in the morning and 102° in the afternoon; on the sixteenth day 100° in the morning and 102° in the afternoon; on the seventeenth day 100° in the morning and 100·8° in the afternoon; on the eighteenth day 99·2° in the morning, and 101° in the afternoon. After this, the temperature, taken once daily until the twenty-second day, was normal.

There are no notes in this case of any symptoms like those of dengue.

Subcutaneous Inoculation of Blood, taken on the second day of the Mosquito-conveyed Disease (Mosquito Case No. III), two days outside the body. *Result*: positive. *Incubation period*: five days twenty-one hours.

Case 29. *P. S., m.*, 46, was injected subcutaneously on 24. v. 16 at 3 p.m. with 1 c.c. of blood from W. (Mosquito Case No. III), taken on the second day of illness (22. v. 16).

The patient states he felt unwell, feeling drowsy and having aching eyes, about 1 p.m., on 29. v. 16. He took to his bed about 6 p.m. on 30. v, his temperature being 101° F.

31. v. 16. He complains of pain in the head, back, thighs and hips, and sleeplessness. On examination: the face was slightly flushed; the eyes injected; the tongue coated; no coryza; no cough; no rash but a congestion erythema of the back. Temperature, 8 a.m., 100°; 4 p.m., 101°; midnight, 100·4°.

1. vi. Marked headache; aching eyes; pains all over the body; the pharynx injected; slight cough; a pinkish raised erythematous mottling, irregularly arranged around little islets of white, was copious on the back, chest, abdomen, buttocks, thighs and shoulders. Temperature, 4 a.m., 99·8°; 8 a.m., 101·2°; noon, 102·2°; 4 p.m., 102·8°; 8 p.m., 102°; midnight, 100·6°.

2. vi. No symptoms; tongue coated; cough; vomiting during the previous night. Rash the same as on the previous day. Temperature, 4 a.m., 99·3°; 8 a.m., 101·2°; noon, 100°; 8 p.m., 99·8°; midnight, normal.

3. vi. Feels well; profuse sweating during the previous night; rash still copious

on the back, chest, shoulders, abdomen and thighs. Temperature, 8 a.m., 98·8°; 4 p.m., 99·4°; midnight, normal.

4. vi. Feels weak; rash fading except on the shoulders. Temperature normal.

5. vi. Feels well; rash has disappeared except from the shoulders. Temperature, normal in the morning, rising to 99° at midnight; after this date it was normal.

6. vi. Still weak. Rash has disappeared except from the shoulders.

7 and 8. vi. Still weak. Rash has disappeared except from the shoulders.

9. vi. Still weak. Rash has disappeared except from the shoulders.

10. vi. Feels well. Rash has disappeared except from the shoulders.

11 and 12. vi. Feels well. Rash has disappeared except from the shoulders. Slight sweating on the night of 11 . vi . 16.

15. vi. Feels well. Rash still on the shoulders, but it was faint on his discharge on this date.

Remarks.

The incubation period was about five days to the first symptom and just over six days to the time he took to bed. The temperature chart is irregular and shews two intermissions. The pulse shews relative bradycardia in the febrile stage and later tends to be slow. The lowest pulse reading was 56. This case was definitely positive.

Subcutaneous Inoculation of Serum and Corpuscles from blood taken on the third day of the Mosquito-conveyed Disease (Mosquito Case V), inoculated the same day, into a subject who had recovered from the inoculated disease (Case 13). *Result*: negative.

Case 30. Case 13, who had reacted positively to dengue material injected on 18. iv. 16, becoming ill on 24. iv, and being discharged on 9. v, was reinjected subcutaneously on 2. vi. with 0·5 c.c. of serum and corpuscles from Mosquito Case No. V ("B. B.") taken the same day, the third day of B. B.'s illness. Although this blood has produced a typical positive result in Case 26, no result followed the re-injection in this case. The temperature was taken once daily for fourteen days. Only on one day (6. vi.) was the temperature above normal, being on that day 99·6° in the morning and 99·2° F. in the afternoon.

The result of the reinjection was negative.

Subcutaneous Inoculation of Serum and Corpuscles from blood taken on the fourteenth day of the Mosquito-conveyed Disease (Mosquito Case No. V), outside the body several hours. *Result*: negative.

Case 31. *G. R.*, *m.*, 49, was injected subcutaneously on 13. vi. 16 with eight minims of serum and corpuscles from Mosquito Case No. V ("B. B."). This blood had been taken the same day, the fourteenth day of B. B.'s illness. No symptoms followed. The case was under observation at least nine days and the temperature taken once daily did not rise above the normal.

Editorial Note

Of the numerous charts sent by the authors a limited number have been reproduced, typical ones being selected. Owing to their absence on military service the authors were unable to pass the proofsheets of this publication.

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